2015

A Novel Function for FANCJ Helicase In Microsatellite Stabilization During Replication Stress

Joanna Barthelemy
Wright State University

A NOVEL FUNCTION FOR FANCJ HELICASE IN MICROSATELLITE STABILIZATION DURING REPLICATION STRESS

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

By

JOANNA ROSE BARTHELEMY
B.S., Daemen College, 2010

2015
Wright State University

WRIGHT STATE UNIVERSITY

GRADUATE SCHOOL

December 14, 2015

I HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER MY SUPERVISION BY Joanna Rose Barthelemy ENTITLED A novel function for FANCJ helicase in microsatellite stabilization during replication stress BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Doctor of Philosophy.

Michael Leffak, Ph.D.
Dissertation Director

Mill W. Miller, Ph.D.
Director, Biomedical Sciences
Ph.D Program

Robert E. W. Fyffe, Ph.D.
Vice President for Research and
Dean of the Graduate School

Committee on
Final Examination

Michael Leffak, Ph.D.

Steven J. Berberich, Ph.D.

Thomas L. Brown, Ph.D.

Courtney E. W. Sulentic, Ph.D.

Yongjie Xu, Ph.D.

ABSTRACT

Barthelemy, Joanna Ph.D., Biomedical Sciences Ph.D. program , Wright State University, 2015. A novel function for FANCJ helicase in microsatellite stabilization during replication stress.

Noncanonical DNA structure-forming sequences, such as hairpin structures, stall replication forks *in vivo*, expand or contract during DNA replication, and colocalize with chromosome fragile sites. Since the frequency of hairpin formation is higher than the frequency of expansion or contraction, the cell may possess mechanisms to resolve hairpin structures prior to replication fork stalling. One possible activity for hairpin resolution is the FANCJ DNA helicase, known to unwind noncanoncial DNA structures *in vitro*. Indeed, mutations or loss of any of the nineteen FANC genes, including FANCJ, leads to genome instability and the cancer-prone syndrome Fanconi anemia (FA).

To test whether $(CTG)_n \cdot (CAG)_n$ trinucleotide repeat (TNR) stability is protected by FANCJ in human cells, HeLa derived cell lines were created by integration of cassettes containing the c-myc replication origin and $(CTG)_n \cdot (CAG)_n$ TNRs. Small pool PCR was used to assess genome instability after siRNA depletion of FANCJ helicase in the presence of a replication stress. The results of these experiments indicate FANCJ helicase is essential for the maintenance of $(CTG)_n \cdot (CAG)_n$ TNR stability independent of replication polarity, and DNA double strand breaks (DSBs) occur near $(CTG)_n \cdot (CAG)_n$ microsatellites in the absence of FANCJ helicase. These results were confirmed in FA patients cells null for FANCJ. Remarkably, several other types of microsatellite sequences capable of forming noncanoncial DNA structures (hairpins, triplexes, G-quadruplexes, unwound DNA) also suffered DSBs in FANCJ depleted cells, indicating a novel role for FANCJ in microsatellite stabilization across the genome. In contrast, HeLa

cells depleted for other FANC proteins or patient cells null for other FA proteins did not show a similar phenotype suggesting FANCJ stabilizes microsatellite sequences apart from activation of the FA repair pathway.

Genome instability present at microsatellite sequences results in loss of small pool PCR signal leading us to believe double strand break formation has occurred. We suspect loss of PCR signal results from unrepaired DSBs or DSBs repaired by a translocation event to another site along the genome. To understand the mechanism of double strand break repair at the ectopic site (CTG)·(CAG) trinucleotide repeats, determined by small pool PCR signal loss, inverse PCR coupled with next generation sequencing was employed. The results of next generation sequencing revealed that DSBs are occurring at or near the (CTG)·(CAG) repeats of the ectopic site in cells depleted of FANCJ and treated with aphidicolin lead to translocations with other sites along the genome. Next generation sequencing identified chromosomal positions of translocation sites. These chromosomal positions correspond to cytogenetic positions which have previously been identified as being associated with chromosome breaks in tumors and cells from patients with several developmental disorders. These results suggest a role for FANCJ helicase in cancer and other developmental diseases as a protector of microsatellite stability.

Table of Contents

List of Figures

List of Tables

ACKNOWLEDGEMENTS

With the sincerest of thanks, I would like to first recognize my mentor Dr. Michael Leffak for providing help, guidance and an exceptional training environment, which have been invaluable in my development into a successful scientist. I am grateful for the wisdom and freedom he offered me as I navigated through this journey. I am truly thankful for his support in and out of the lab. I would also like to thank the members of my dissertation committee, Dr. Thomas Brown, Dr. Steven Berberich, Dr. Courtney Sulentic and Dr. Yongjie Xu, for their invaluable comments and suggestions.

To the past and present members of the Leffak laboratory, thank you for intellectual conversations and your friendship; you made coming to work an enjoyable adventure every day. I would like to thank the entirety of the Biochemistry & Molecular Department for constant encouragement and support, both tangible and intangible. Additionally, a special thanks the Biomedical Sciences Program for providing me with an opportunity to grow as a scientist.

Lastly, but certainly not least a special thank you to my family and friends. Specifically, I will forever be grateful to Natasha Hill and Richard Pye for their friendship, which made this journey a little bit easier to endure. To my family, thank you for your continued support, love and encouragement throughout this crazy endeavor. Words will never express how lucky I am to have you on my side.

DEDICATION

To my parents, with love

"My world is a better place because of you"

-Celine Dion, Because you loved me

INTRODUCTION

(CTG)·(CAG) trinucleotide repeats are dynamic DNA sequences

Microsatellites, short repetitive DNA sequences (such as (CTG)·(CAG) repeats), are prone to form noncanonical DNA structures such as hairpins, triplexes, and G-quadruplexes when DNA is in a single strand conformation (Figure 1) (Collins et al., 2003; Cooper et al., 2011; Gatchel and Zoghbi, 2005; McMurray, 2010). These structures pose a threat to proper DNA replication, represent hotspots for chromosomal breakage and translocations, and increase the potential for genome instability (Cooper et al., 2011; Gatchel and Zoghbi, 2005; McMurray, 2010; Zeman and Cimprich, 2014). During DNA metabolism, the structure forming sequence is exposed after unwinding from histones leading to the formation of non-B DNA structures. The noncanonical DNA structure is susceptible to DNA damage and evades DNA repair mechanisms resulting in accumulation of DNA damage (Aguilera and Gomez-Gonzalez, 2008; Glover et al., 1984; Wang and Vasquez, 2009). Models for noncanonical DNA structures to accumulate double strand breaks during replication are depicted in Figure 2 (Aguilera and Gomez-Gonzalez, 2008; Glover et al., 1984). Expansion or contraction in the number of repeats of the microsatellite and formation of double strand breaks at or near the repetitive sequence represent consequences of noncanoncial DNA structure formation and are termed instability (Zeman and Cimprich, 2014). All forms of repeat instability

threaten genomic integrity. Genomic aberrations caused by instability of microsatellites are associated with multiple human diseases.

(CTG)·(CAG) repeat instability association with DM1

(CTG)·(CAG) trinucleotide repeats are one subset of microsatellite sequences. (CTG)·(CAG) repeat instability has been linked to multiple neurodegenerative disorders including myotonic dystrophy type1. Myotonic dystrophy type 1 (DM1) refers to a chronic, systemic disorder affecting approximately 1 in 8,000 individuals worldwide (Shaw and Harper, 1989). Characteristics of DM1 include muscle wasting, myotonia (prolonged muscle contractions), cataracts, heart conduction defects, and weakness in the extremities and face (Shaw and Harper, 1989). The cause of DM1 has been linked to an expansion of a (CTG)·(CAG) microsatellite, a short repeated DNA sequence, in the dystrophia myotonica protein kinase (DMPK) gene (Fernandez-Lopez et al., 2004; McMurray, 2010). (CTG)·(CAG) repeat lengths are categorized into three classes: normal range (5-37 repeats), pre-mutation (38-49 repeats), and symptomatic disease (≥50 repeats). In affected individuals there is a direct correlation between the length of the (CTG)·(CAG) repeats and severity of the disease, and an inverse correlation with the age of disease onset, termed genetic anticipation. The (CTG)·(CAG)repeats are located in the 3’ untranslated region (3’ UTR) of the DMPK gene (Brook et al., 1992). An expansion in the (CTG)·(CAG) repeats leading to a disease state and the observed phenotypes results from an accumulation of unprocessed messenger RNA (mRNA) in the nucleus (Roberts et al., 1997). The large number of (CTG)·(CAG) repeats transcribe into CUG repeats (Brook et al., 1992; Tiscornia and Mahadevan, 2000). These CUG repeats sequester splicing proteins, specifically musclebind like protein (MBNL), affecting the proper

processing of other pre-mRNAs (Tiscornia and Mahadevan, 2000). Recent research suggests prevention of proper mRNA processing through altered MBNL levels directly correlates to myotonia, muscle wasting, and cardiac abnormalities seen in DM1 patients (Orengo et al., 2008).

Our laboratory previously showed the (CTG)·(CAG) repeats responsible for DM1 form hairpin structures *in vivo* (Liu et al., 2010). Replication forks move slower and hold a greater tendency to stall at fragile sites, such as (CTG)·(CAG) repeats (Freudenreich et al., 1997; Miret et al., 1998; Pelletier et al., 2003). Therefore, understanding the mechanisms necessary to resolve hairpin structures is of importance.

$(CTG)_n$·$(CAG)_n$ DM1 model cell lines

To examine phenotypes of the active (CTG)·(CAG) repeats, we created HeLa derived cell lines containing a single ectopic copy of (CTG)·(CAG) repeats (representative of normal (12 repeats), pre-mutation (45 repeats), and disease state (102 repeats)) next to the c-myc replication origin to mimic replication across the (CTG)·(CAG) repeats at the endogenous DMPK locus (Liu et al., 2010; Liu et al., 2012). The c-myc replicator acts as a point of reference to map the direction of replication. The orientation of the (CTG)·(CAG) repeats relative to the c-myc replicator determines which repeat (CTG or CAG) becomes the template for leading strand synthesis and the complementary repeat is the template for lagging strand synthesis (Liu et al., 2010). This model system helped demonstrate that hairpins form during replication, suggesting that hairpins can form on either the template DNA or nascent DNA during replication and unresolved hairpins present in the template DNA can lead to contractions while hairpins

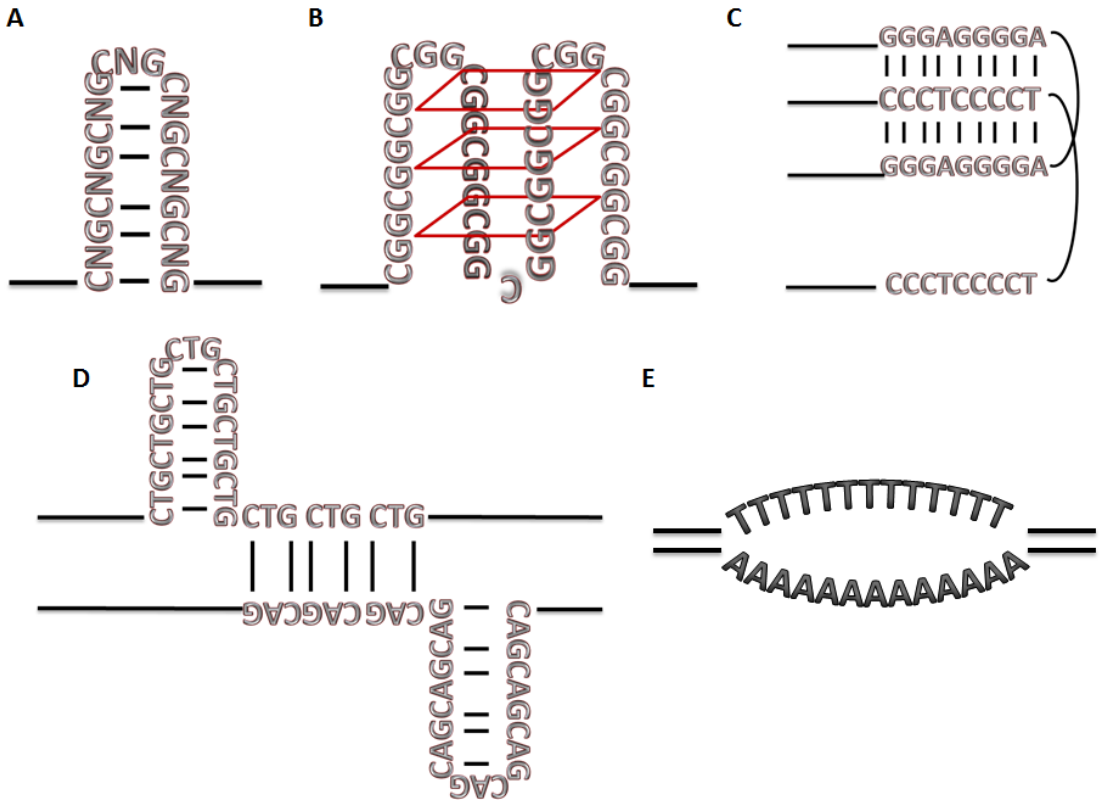

Figure 1: Possible non-B form structures formed at microsatellites during replication. **(A-D)** Schematics of unusual DNA structures formed by repeated sequences. (CTG)·(CAG) repeats fold into hairpin structures **(A)** in vivo, DNA fitting the G4 consensus sequence folds into G-quadruplex structures **(B)**, slip strand structures can occur at trinucleotide repeats **(D)**, and polypurine·polypyrimidine sequences can fold back onto itself creating a triplex **(C)** when DNA is in a single strand conformation. (Adapted from Cooper et al., 2011)

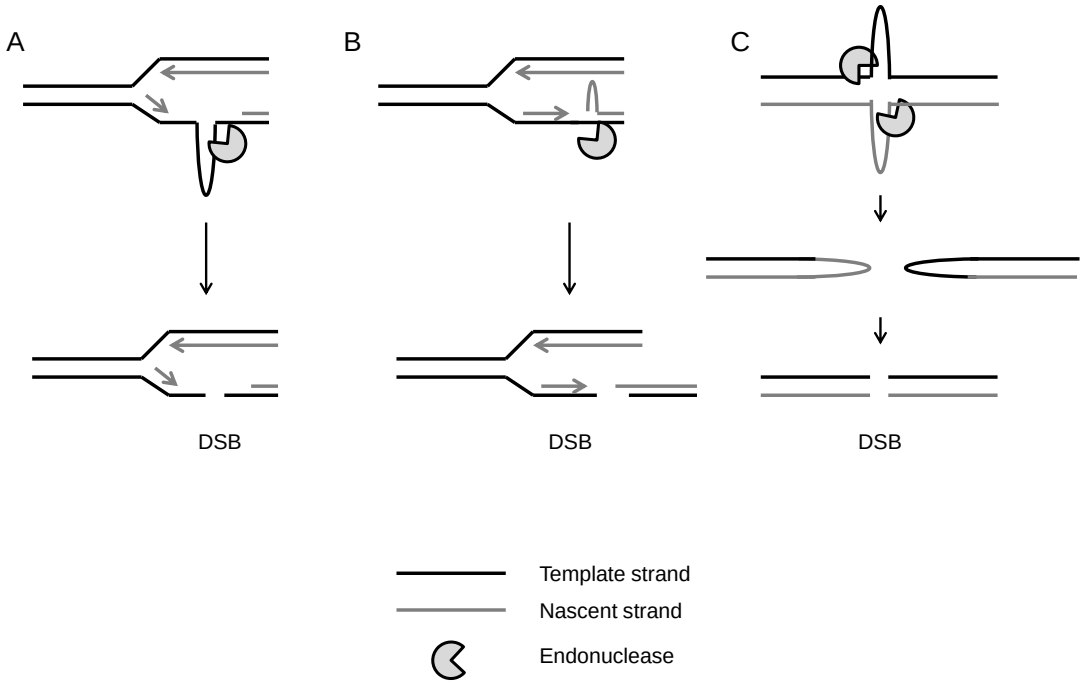

Figure 2. Mechanisms for double strand break formation at repetitive DNA sequences. **(A)** Hairpin structures are substrates for DNA repair endonucleases, which create nicks in the DNA at the base of the hairpin. The nicked DNA is then converted to a double strand break (DSB). **(B)** Hairpin structures can form on the 5' end of an Okazaki fragment preventing the activity of the flap endonuclease FEN1 facilitating a cut on the template strand leading to double strand break (DSB) formation. **(C)** Hairpin structures can form in both the template (black) and daughter strand (grey) creating a Holliday junction structure which will be converted to two hairpin ended molecules that will be further processed by repair proteins into a double strand break (DSB). Although hairpins are represented in this figure, other noncanonical DNA structures including triplexes and G-quadruplexes would be processed in the same manner. (Adapted from Aguilera and Gomez-Gonzalez, 2008)

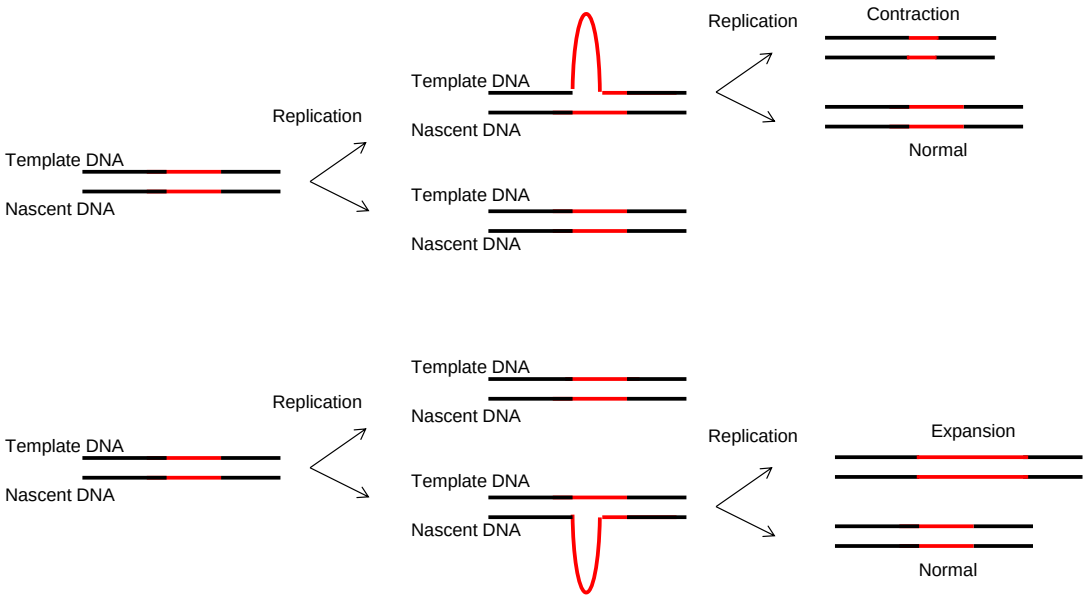

Figure 3: Hairpin-induced trinucleotide repeat instability. Using HeLa derived cell lines containing a single copy of (CTG)·(CAG) repeats, we showed hairpins form at the trinucleotide repeats during replication and this hairpin formation induces instability. Hairpin formation on the template strand during replication causes contractions while hairpin formation on the nascent strand during replication produces expansions. (Adapted from Liu et al., 2010)

present in the nascent DNA result in expansions (Figure 3) (Liu et al., 2010). Both contractions and expansions represent potentially harmful forms of genome instability (Liu et al., 2012). The mechanism necessary for resolving hairpins and maintaining genome stability at the (CTG)·(CAG) repeats of the DMPK locus remains unknown.

Since the frequency of hairpin formation is much higher than the frequency of expansion or contraction, the cell likely possesses mechanisms to resolve hairpin structures and avoid replication fork stalling. Possible enzymes for hairpin resolution are DNA helicases known to unwind noncanonical DNA structures. Genome instability disorders arise from mutations or loss of these helicases including the cancer predisposition diseases Fanconi anemia (FANCJ helicase) (Litman et al., 2005), Bloom's syndrome (BLM helicase) (Ellis et al., 1995), Werner's syndrome (WRN helicase) (Yu et al., 1996), and Rothmund-Thomson syndrome (RecQL4 helicase) (Kitao et al., 1999).

Fanconi anemia (FA) and genome instability

Many different genome instability diseases exist. FA refers to an autosomal recessive disorder characterized by bone marrow failure, developmental abnormalities, and cancer predisposition (Deakyne and Mazin, 2011; Kee and D'Andrea, 2010; Niedernhofer et al., 2005). All FA patients possess severe chromosomal instability (Deakyne and Mazin, 2011; Niedernhofer et al., 2005). Mutations in one of nineteen different FA complementation groups, each complementation group represents mutations in a single FANC protein expressing gene, leads to hypersensitivity to interstrand crosslinking agents. Initial work categorized a group of eight of genetic complementation

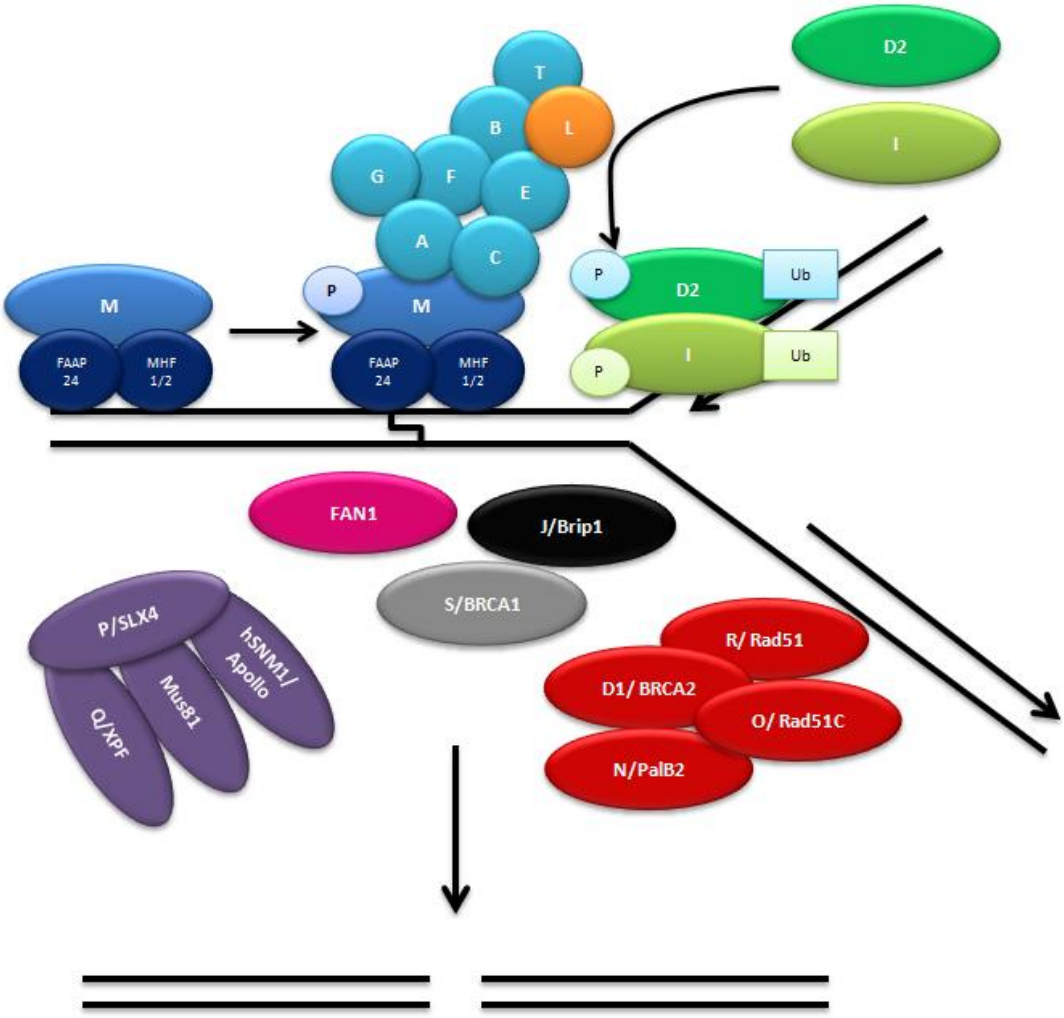

Figure 4: Fanconi anemia repair pathway. A schematic of the assembly of FANC proteins to the site of an inter-strand crosslink for DNA damage repair.

groups corresponding to a cluster of FANC proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, FANCM) that aggregate together in response to damaged DNA (Deakyne and Mazin, 2011; Raschle et al., 2008; Xue et al., 2008). Recently, an additional core complex protein was identified, the ubiquitin E2 conjugase UBE2T (FANCT) (Rickman et al., 2015; Virts et al., 2015). After a DNA damaging event, DNA damage sensing kinases (ATR and ATM) phosphorylate FANCM resulting in the formation of the FA core complex consisting of these nine FANC proteins (Raschle et al., 2008; Shigechi et al., 2012). As an E3 ubiquitin ligase, FANCL functions to monoubiquitinate both phosphorylated FANCI and phosphorylated FANCD2 leading to FANCI/FANCD2 heterodimerization and translocation of both proteins to the site of DNA damage creating nuclear foci (Garner and Smogorzewska, 2011; Joo et al., 2011; Raschle et al., 2008). Subsequently, additional FA proteins travel to the site of DNA damage including FANCD1 (BRCA2) (Wagner et al., 2004), FANCN (PALB2) (Tischkowitz and Xia, 2010), activated (phosphorylated) FANCJ (Levitus et al., 2005), BRCA1 (FANCS) (Sawyer et al., 2015), FANCP (SLX4) (Stoepker et al., 2011), FANCQ (ERCC4) (Bogliolo et al., 2013), FANCR (RAD51) (Bogliolo and Surralles, 2015; Wang et al., 2015a), and FANCO (RAD51C) (Somyajit et al., 2012). Once assembled at the site of damaged DNA, DNA repair is initiated (Figure 4). In addition to resolution of interstrand crosslinks, members of the FA repair pathway are required for normal DNA replication and the response to replication stress. Therefore, many FA proteins operate in other DNA repair pathways including nucleotide excision repair (NER), mismatch repair (MMR), and homologous recombination (HR) (Kee and

D'Andrea, 2010). Of interest for this work is the function of the FANCJ helicase, which acts in a number of these replication dependent repair processes.

FANCJ and genome instability

Fanconi anemia completion group J patients share classification as a result of mutations within the FANCJ gene (Wu et al., 2008). FANCJ was first identified as a binding partner of the breast cancer type 1 protein (BRCA1) and was referred to as BRCA1-interacting protein 1 (Brip1) (Cantor et al., 2001; Greenberg et al., 2006). Further examination of the interaction between FANCJ and BRCA1 revealed that FANCJ binds to the BRCA1 C-terminal (BRCT) repeat motif found within the C-terminus of BRCA1 binding partners (Cantor et al., 2001). As previously stated, a subset of FA patients have mutations within the FANCJ gene and it was later determined FANCJ is Brip1 (Litman et al., 2005). The FANCJ gene codes for a helicase functioning to unwind DNA in a 5' to 3' orientation (i.e. FANCJ moves 5' to 3' along a DNA strand) (London et al., 2008). FANCJ unwinds noncanonical DNA structures such as G-quadruplexes (Figure 1b) and DNA repair intermediate structures such as Holliday junctions (London et al., 2008; Wu et al., 2008). Functioning with the FA repair pathway, FANCJ plays a role in the protection of genome stability in response to interstrand crosslinking reagents (Wu et al., 2008; Wu et al., 2009). Recently, the FANCJ analog in *C. elegans*, dog-1 (Youds et al., 2008), was shown to be crucial for genome stability (Cheung et al., 2002; Kruisselbrink et al., 2008; Wu et al., 2009). The loss of dog-1 lead to the deletion of G4 DNA, DNA known to fold into G-quadruplex structures, and this deletion began immediately 5' of the start of the G4 DNA and ended at random locations hundreds of nucleotides downstream of the G4 DNA (Cheung et al., 2002; Kruisselbrink et al., 2008).

Similar results were seen in chicken cells null for FANCJ (Kitao et al., 2011). The human homolog of FANCJ interacts with multiple DNA damage responding proteins including BRCA1, the repair nuclease Mre11, the replication and repair protein TopBP1, the single strand DNA binding protein RPA, the mismatch repair protein MLH1, and the BLM helicase. Blocking the interaction of FANCJ with these proteins leads to reduced cell survival under replicative stress. Additionally, mutations resulting in loss of FANCJ display similar cellular phenotypes when exposed to replication stress. Recent work has indicated a role for FANCJ in DNA replication since FANCJ patient cells are hypersensitive to DNA polymerase inhibitors and interstrand crosslinking reagents (Levitus et al., 2005; Levran et al., 2005; Litman et al., 2005). Taken together; these results suggest an essential role for FANCJ in the maintenance of genome stability through a known function of unwinding noncanonical DNA structures. However, the exact role FANCJ plays in the protection of genome stability in human systems remains unknown. Thus, the work described below focuses on understanding the function of FANCJ in microsatellite stabilization.

MATERIALS AND METHODS

Cell Culture

Cell lines were derived from a HeLa acceptor cell line containing a single FRT (FLP recombinase target) site (HeLa/406 acceptor cell line) on chromosome 18 (Liu et al., 2003). Acceptor cells were co-transfected with a plasmid expressing FLP recombinase and a donor plasmid containing (CTG)·(CAG) repeats downstream of the c-myc replicator (integration of the donor plasmid at the FRT site in acceptor lines is outlined in Figure 5). Donor plasmids are designed such that the direction of replication from the c-myc origin through the repeats is known allowing for identification of which repeat (CTG or CAG) acts as leading strand replication template and lagging strand template for replication. We developed four different cell lines containing varying numbers of (CTG)·(CAG) repeats representing normal and disease states. Each repeat was placed next to the c-myc replicator in both the forward and reverse orientation. This resulted in two separate cell lines for each number of repeats, the repeat (CTG or CAG) is the template for leading strand synthesis during replication in one cell line and the template for lagging strand synthesis in the second cell line. Cell lines were named based on the repeat present in the lagging strand template (Figure 6). $(CTG)_{102}/(CAG)_{102}$ (disease state) cells will be used in all the following experiments and $(CTG)_{12}/(CAG)_{12}$ (normal/unaffected) cells will serve as controls, this short repeat length should not be susceptible to genome instability. FANCJ null (752) and 752 cells complemented with

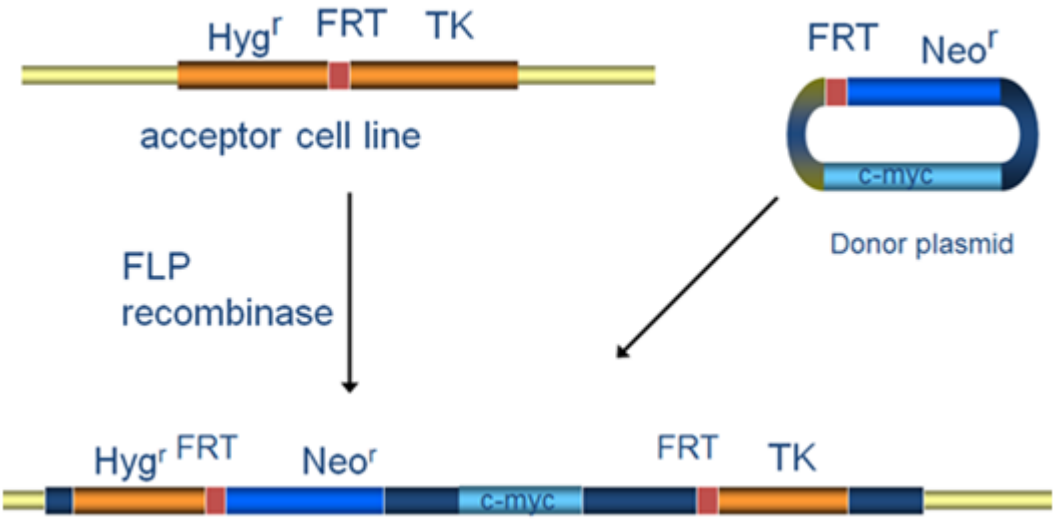

Figure 5: Chromosomal integration of c-myc-(CTG)·(CAG) constructs. A diagram of stable integration of donor plasmids containing the c-myc replicator adjacent to varying lengths of (CTG)·(CAG) repeats to create DM1 model cell lines.

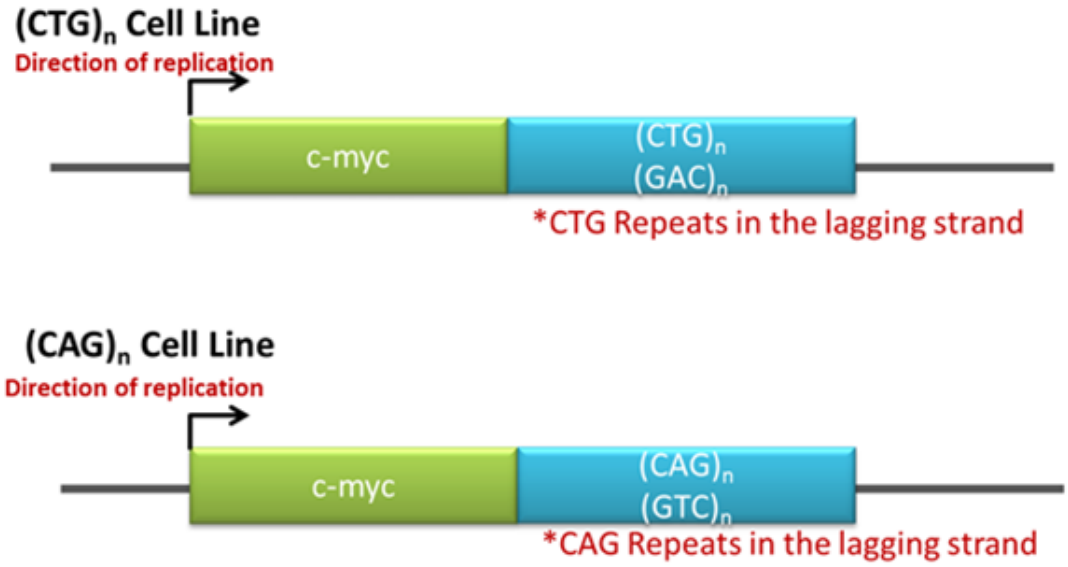

Figure 6: Schematic of donor c-myc-(CTG)·(CAG) donor plasmids. A cartoon representation of (CTG)·(CAG) donor plasmids. (CTG)·(CAG) repeats are positioned downstream of c-myc replicator. Cell lines are termed based on the repeat (CTG or CAG) present in the lagging strand (as indicated in red).

wild type FANCJ (752+FANCJ WT) (Raghunandan et al., 2015), FANCC, FANCQ, and FANCI immortalized Fanconi anemia patient cell lines were generously provided by Dr. Helmut Hanenberg (Heinrich-Heine-Universität Düsseldorf). RA3087 (FANCA), RA3331 (FANCP), RA2645 (FANCD2), RA3100 (FANCL), RA3226 (FANCD1/BRCA2), and RA2374 ($FANCJ^{R798X}$) immortalized Fanconi anemia patient fibroblast cell lines were kindly provided by Dr. Agata Smogorzewska (Rockefeller University) (Kim et al., 2013). Apparently healthy primary fibroblasts (GM08402), Bloom's Syndrome primary fibroblasts (GM166860), SV40 transformed fibroblasts (GM08505), Werner Syndrome primary fibroblasts (AG03141), and SV40 transformed (AG11395) patient fibroblast cell lines were obtained from Coriell Cell Repositories. All cell lines were grown in Dulbeco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum at 37°C and 5% CO_2.

Aphidicolin/Hydroxyurea treatment of fibroblast cell lines

To promote noncanonical DNA structure formation, DM1 model cell lines were treated with reagents known to slow replication progression in conjunction with knock down of proteins of interest. Aphidicolin (APH), a DNA polymerase inhibitor, was used to slow replication (~30-50%) at a concentration (0.2 μM) which will not initiate an acute DNA checkpoint response (Casper et al., 2002; Glover et al., 1984; Wist, 1980). To test whether the results obtained from aphidicolin experiments are drug specific, similar experiments were performed in the presence of low dose (0.3 mM) hydroxyurea (HU), which also slows replication fork movement leading to upregulation of ribonucleotide reductase and firing of dormant replication origins without acutely activating the checkpoint response (Chen et al., 2015; Petermann et al., 2010). In contrast to

aphidicolin, HU is a ribonucleotide reductase inhibitor. Treatment with these replication slowing reagents should enhance possible DNA secondary structure formation at microsatellite sequences. All experiments were performed with and without replication slowing reagents in conjunction with siRNA against proteins of interest or the control siRNA. $(CTG)_n \cdot (CAG)_n$ cells were given 0.2 μM APH or 0.3mM HU for a total of 10 days. Fibroblast cell lines were treated with 0.5 μM APH or 0.3 mM HU for 10 days. Control, untreated counterparts were plated simultaneously. Cells were harvested on the tenth day of treatment using TrypLE ™ Express (Life Technologies 12604), a trypsin alternative.

siRNA Treatment

$(CTG)_n \cdot (CAG)_n$ cells at a confluency of 60% were reversely transfected with siPort NeoFX transfection reagent (Invitrogen 4510M) and 100 nM (final concentration) of small interfering RNA (siRNA) pool (an equal molar mixture of 4 different siRNAs targeting the same transcript) (Dharmacon Research, Inc.) targeting FANCJ/Brip1 (siGENOME SMARTpool M-010587), FANCM (siGENOME SMARTpool M-021955), FANCD2 (siGENOME SMARTpool M-016376), BLM (siGENOME SMARTpool M-007287), or WRN (siGENOME SMARTpool M-010378) in a six-well plate every 48 hours for a total of five transfections in the presence of aphidicolin or hydroxyurea. Cells were harvested using TrypLE ™ Express (Life Technologies) 48 hours post the fifth transfection. Control experiments were carried out in parallel using AllStars negative nontargeting siRNA (Qiagen SI03650318).

PCR

Small pool PCR (spPCR). After cells were treated with siRNA and replication slowing reagents, DNA was isolated from cells using an E.Z.N.A ® Tissue DNA Kit from Omega Bio-Tek (D3396). PCR was performed on a small amount (~1-10 genome equivalents) of DNA template (50 pg of genomic DNA) per reaction. Four small pool PCR (spPCR) reactions were performed on DNA template from each experimental condition. All spPCR reactions occurred in the presence of HotStart Taq polymerase MasterMix (Qiagen 203443) and amplification conditions were 95°C (15 min); 35 cycles of 94°C (1 min), 54°C (45 s), 72°C (1 min); and final extension at 72°C (7 min). PCR products were resolved in 8% polyacrylaminde gels. GelRed (Biotium 41002) stained gels were imaged using a Fuji LAS-3000 and the software ImageReader.

Inverse PCR (iPCR). Samples were derived from CTG_{102} cell DNA treated simultaneously with siFANCJ (5 transfections) and aphidicolin (0.2 μM). DNA (2.5 ug) was digested with an excess of MseI (New England BioLabs, Inc. R0525) for 1 hour at 37°C in a 50μL total volume reaction. After a 20 minute heat inactivation at 65°C, 10 μL of the restriction digest reaction was used for ligation. Ligation occurred in the presence of T4 DNA ligase (New England BioLabs, Inc. M0202) for 1 hour at 16°C. The ligation reaction product was cleaned using the MinElute Reaction Cleanup Kit (Qiagen 28204). Amplification of ligation products occurred using iPCR primers (iPCR forward primer: 5’ CATGCTGTCCAGGCAGGTAGATGA 3’ and iPCR reverse primer: 5’ CCGGAACGCAGAAGAATCATAATGGG 3’) with HotStart Taq polymerase mastermix (conditions: 15 min at 95°C; 35 cycles of 1 min at 94°C, 45s at 58°C, 1 min at

Locus Name	Chromosome Location	Forward Primer	Reverse Primer	Microsatellite Disease associati
Ectopic Site CTG/CAG repeat		AAAAAAGATCCTCTCTCGCTAATCTCCG	GGAAGGAGCTGACTGGGTTGAAGG	
Endogenous DMPK CTG repeat	19q13.3	GAAGGGTCCTTGTAGCCGGGAA	GGAGGATGGAACACGGACGG	Myotonic dystrop type 1
Endogenous SCA1 CTG repeat	6p23	TGGAGGCCTATTCCACTCT	GAGTCGGCGTATTGCATGA	Spinocerebella ataxia type 1
		CCAACATGGGCAGTCTGA	TTTGTCGTTGAGGGTAGAAGG	
Endogenous TCF4 CTG repeat	18q21.1	AATCCAAACCGCCTTCCA	TCTCTTTCACTCCCTCTCTCTC	Fuchs' endotheli corneal dystroph
Endogenous ERDA1 CTG repeat	17q21.3	ATGGATTGTTCCAAGGAG	TTATAACCCTGTCCACTCAGTG	
Endogenous SCA10 ATTCT repeat	22q13.31	TGTAGATAGACTGCAACAAGGTAAA	GAGTGTTGATTAGGGAAGTGATAAATG	Spinocerebella ataxia type 10
Endogenous c-MYC G4 consensus	8q24.21	GGTCGGACATTCCTGCTTTA	CCTCCCTTCGCACTCAATAC	
Endogenous β-globin G4 consensus	11p15.5	CTTTACACAGTCTGCCTAGTACAT	CCAGCCTTATCCCAACCATAA	
Endogenous α-1b tubulin G4 consensus	12q13.12	CCCAACCTACACTAACCTTAACC	CCACACCAACCTCCTCATAAT	
		CTCTTCCGCAGTCCTCTTATTG	GAGACCTCGGTGTGTGTAAATAG	
Endogenous PKD1 Py·Pu mirror repeat	16p13.3	CTCCTCTTCTTCTCCCTTCTCT	GATTGGAGTCCACCAGAAAGAT	Polycystic kidne disease type 1
Endogenous p63 poly T repeat	3q28	CTGGAGTTCGGCAGTACAATCTCA	ATTATTTGCTAAATGCGTAAGTGAA	
Endogenous p53 poly T repeat	17p13.1	GGTTGAGTCTCCAAAGTCTTCA	AAATCCCGCGACAGCAA	
Endogenous BRIP1 polyT repeat	17q22.2	TCCTTATCCTCTACCTCTAGCC	GATAGCTGGGCTCTCCATTT	
Internal Control (250 bp)	18	GTAGGTTCAAAGGGTGGGT	GGCAACGTGACAAGGAATG	
Internal Control (335bp)	19q13.3	CTCACTGGTCACTGGTTTCTT	CCAACCCAACTTCATCCTCTAC	
Internal Control (97 bp)	18	CTTTGTGGCTGAAGGACTGA	ACGTGACAAGGAATGCAGAT	
Ectopic Site Upstream		TGCTCCTGCCGAGAAAGTATCCAT	CTTCAGCAATATCACGGGTAGCCAAC	
Ectopic Site Downstream		GATTAGCAGAGCGAGGTATGTAG	GAGTCAGGCAACTATGGATGAA	

Table 1. Small pool PCR primer pairs used in this work. Primer sets used for spPCR to examine instability of repeated sequences and internal controls.

72°C; 7 min at 72°C). iPCR products were cleaned using the MinElute Reaction Clean-up (Qiagen 28204) and used for next generation sequencing.

A list of primers used in this work can be found in Table 1.

Western Blotting

Whole cell lysates from treated or untreated HeLa cells and fibroblast cells were prepared with Mammalian Protein Extraction Reagent (M-PER) (Thermo Scientific 78501) and protease inhibitor cocktail (Sigma P8340). After SDS-PAGE and transfer to a polyvinylidene fluoride (PVDF) membrane (ThermoFisher Scientific 88518), membranes were probed using a 1:500 dilution of primary antibody (rabbit FANCJ/Brip1 (Abcam ab151509), mouse FANCM (Dr. Lei Li, University of Texas MD Anderson Cancer Center) (Wang et al., 2013), mouse FANCD2 (Santa Cruz Biotechnology, Inc. SC-20022), rabbit BLM-7099 (Dr. Jan Karlseder, The Salk Institute for Biological Studies) (Orazio et al., 2011), mouse WRN (Abcam ab66606) and mouse β-actin (Sigma-Aldrich A5441)) overnight at 4°C. After incubation with the corresponding horseradish peroxidase (HRP) conjugated secondary antibody at a dilution of 1:1000 at room temperature for 1 hour, membranes were imaged on a Fuji LAS-3000 using ImageReader.

Aphidicolin Sensitivity

Using a reverse transfection approach, CAG_{102} cells were transfected with FANCJ or control siRNA every 48 hours. Continuous aphidicolin (0.2 μM) treatment began 24 hours post the first transfection (0 hr) for a total of 96 hours. Fibroblast cells were treated continuously with aphidicolin (0.5 μM) for 96 hours. Treated cells were harvested using

TrypLE ™ Express (Life Technologies) and viable cells were counted every 24 hours using trypan blue (Sigma-Aldrich T8154) exclusion.

Flow Cytometry

FANCJ null fibroblast cells were treated with 0.5 μM aphidicolin for a total of 10 days. Treated cells were collected and fixed in cold 70% ethanol for 10 minutes at room temperature. After washing with PBS and 5mM EDTA (3X; 15 minutes per wash), cells were incubated with propidium iodide (PI) (Sigma-Aldrich P4170) for 30 minutes. Stained cells were analyzed by flow cytometry.

Next Generation Sequencing

The Case Western Reserve University Genomics Care facility performed next generation sequencing analysis on iPCR products of DNA from CTG_{102} cells treated with FANCJ siRNA and aphidicolin.

RESULTS

I. FANCJ is essential for microsatellite stabilization

FANCJ is necessary for CTG_{102}·CAG_{102} trinucleotide repeat stability

Through interaction with multiple proteins known to respond to noncanonical DNA structures and replication stress, FANCJ must play a critical role in DNA replication. Noncanonical DNA structures known to slow replication forks include CTG·CAG hairpins, G-quadruplexes, and asymmetric purine·pyrimidine (Pu·Py) repeat tracts able to form DNA triplexes (H-DNA). Previous work has shown that $(CTG)_{102}$·$(CAG)_{102}$ microsatellites form hairpins *in vivo* and stall replication forks (Liu et al., 2010). Since FANCJ unwinds DNA structures responsible for inhibition of DNA replication and $(CTG)_{102}$·$(CAG)_{102}$ repeats form hairpin structures *in vivo*, we wanted to examine the effect of loss of FANCJ helicase on $(CTG)_{102}$·$(CAG)_{102}$ repeat stability.

HeLa-derived cell lines harboring a single ectopic copy of CTG_{102}·CAG_{102} trinucleotide repeats (TNRs) directly downstream of the c-myc core replicator were created (Figure 7A). During replication, the CTG_{102} or CAG_{102} repeat serves as lagging strand template in its respective cell line. A pool of siRNAs was used to knockdown FANCJ (Figure 7B) in the absence (control) or presence of a low dose of aphidicolin (0.2

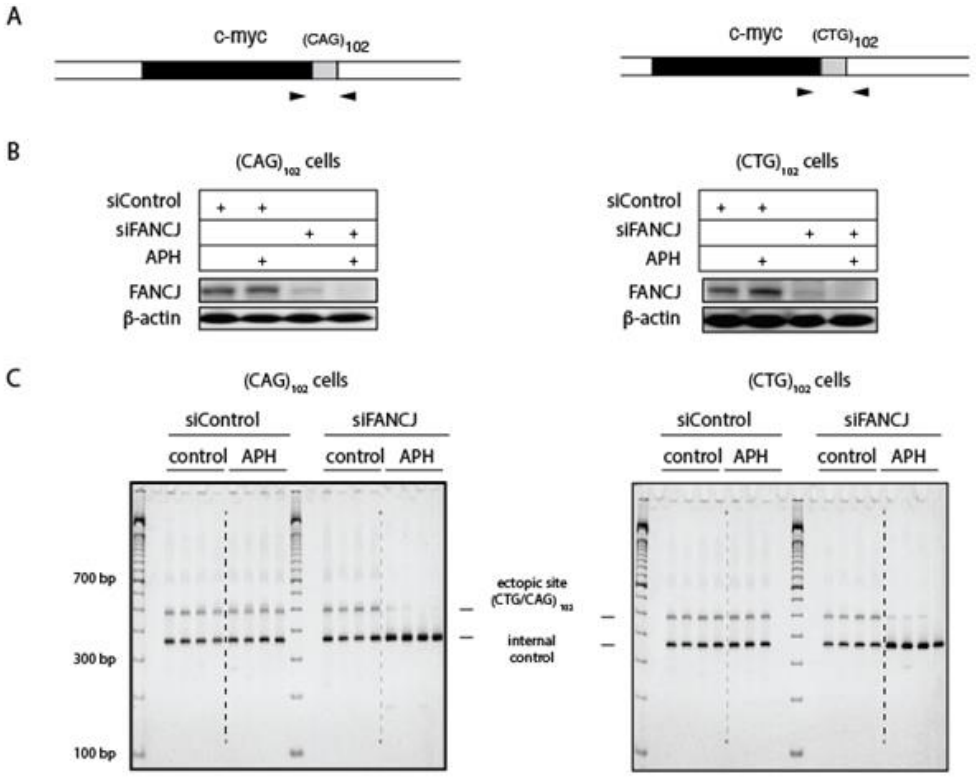

Figure 7. FANCJ knockdown leads to loss of ectopic (CTG)·(CAG) microsatellite signal in $(CTG)_{102}$·$(CAG)_{102}$ cells treated with aphidicolin. **(A)** Diagram of the integration site in $(CAG)_{102}$·$(CTG)_{102}$ cell lines. **(B)** Whole cell extracts were isolated after treatment of cells with siRNA against FANCJ and aphidicolin for a total of five transfections, or parallel untreated cultures, and immunoblotted for FANCJ. **(C)** Small pool PCR results with primers flanking the ectopic $(CAG)_{102}$·$(CTG)_{102}$ repeats and primers for a site without microsatellites, an internal control for proper function of the PCR machinery.

μM APH) that slows DNA polymerases (~30-50%) and reveals chromosome fragile sites without activating the DNA checkpoint response in CTG_{102}·CAG_{102} cells (Casper et al., 2002; Glover et al., 1984; Wist, 1980). Non-targeting siRNA was used as a control. Duplex small pool PCR (spPCR) analysis of genomic DNA was performed with an internal control primer set to assess differences between cells in the population and detect expansions, contractions, or breaks (instability) of the microsatellite sequences. spPCR results from CTG_{102} and CAG_{102} cells showed a dramatic decrease in PCR signal at the ectopic site (CTG)·(CAG) repeats in CTG_{102}·CAG_{102} cells after loss of FANCJ in the presence of APH (Figure 7C). However, spPCR of sequences 1-3 kb upstream or downstream of the (CTG)·(CAG) repeats of the ectopic site did not exhibit instability (Figure 8). In addition to CTG_{102}·CAG_{102} cells, CTG_{12}·CAG_{12} cells were constructed. Twelve repeats are indicative of a normal range of repeats. Similar to the CTG_{102}·CAG_{102} cells, CTG_{12}·CAG_{12} cells were depleted of FANCJ using siRNA (Figure 9B) and treated with APH. Surprisingly, we observed in cells treated with both siFANCJ and APH, PCR signal of the (CTG)·(CAG) ectopic site repeats was diminished compared to control cells (Figure 9C) suggesting FANCJ is essential for (CTG)·(CAG) trinucleotide repeat stability even at nonpathological repeat lengths.

As a control to link genome instability to the (CTG)·(CAG) trinucleotide repeats at the ectopic site, we created an additional HeLa derived cell line in which the (CTG)·(CAG) repeats were replaced with a nonrepetitive sequence at the integration site (Figure 10A). Depletion of FANCJ in the presence of aphidicolin in these cells did not result in microsatellite instability at the ectopic site (Figure 10B) confirming

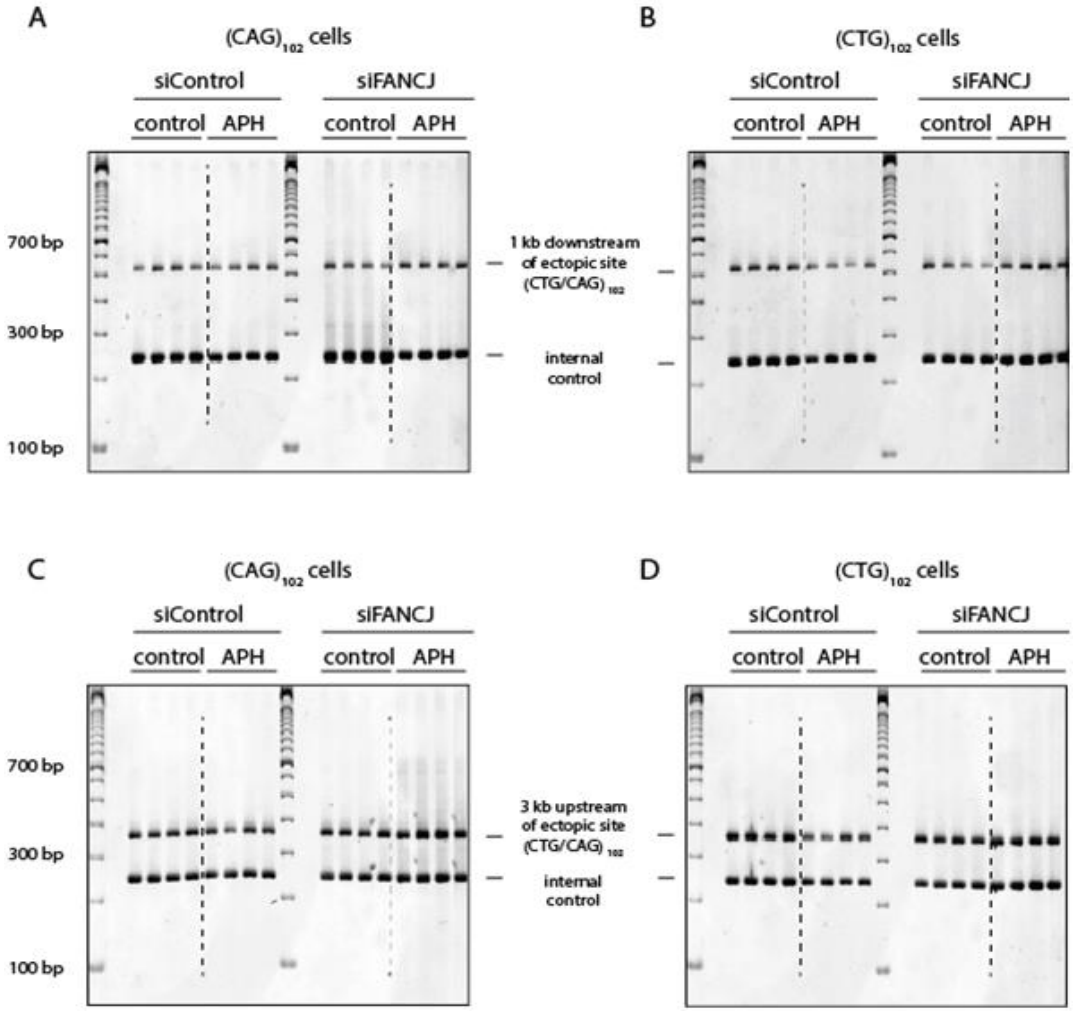

Figure 8. FANCJ knockdown leads does not lead to spPCR signal loss 1-3kb upstream and downstream of (CTG)·(CAG) repeats. **(A, B)** Small pool PCR results with primers ~1kb upstream of the ectopic (CTG)·(CAG) repeats and primers for an internal control. **(C, D)** Small pool PCR results with primers ~3kb downstream of the ectopic (CTG)·(CAG) repeats and primers for an internal control.

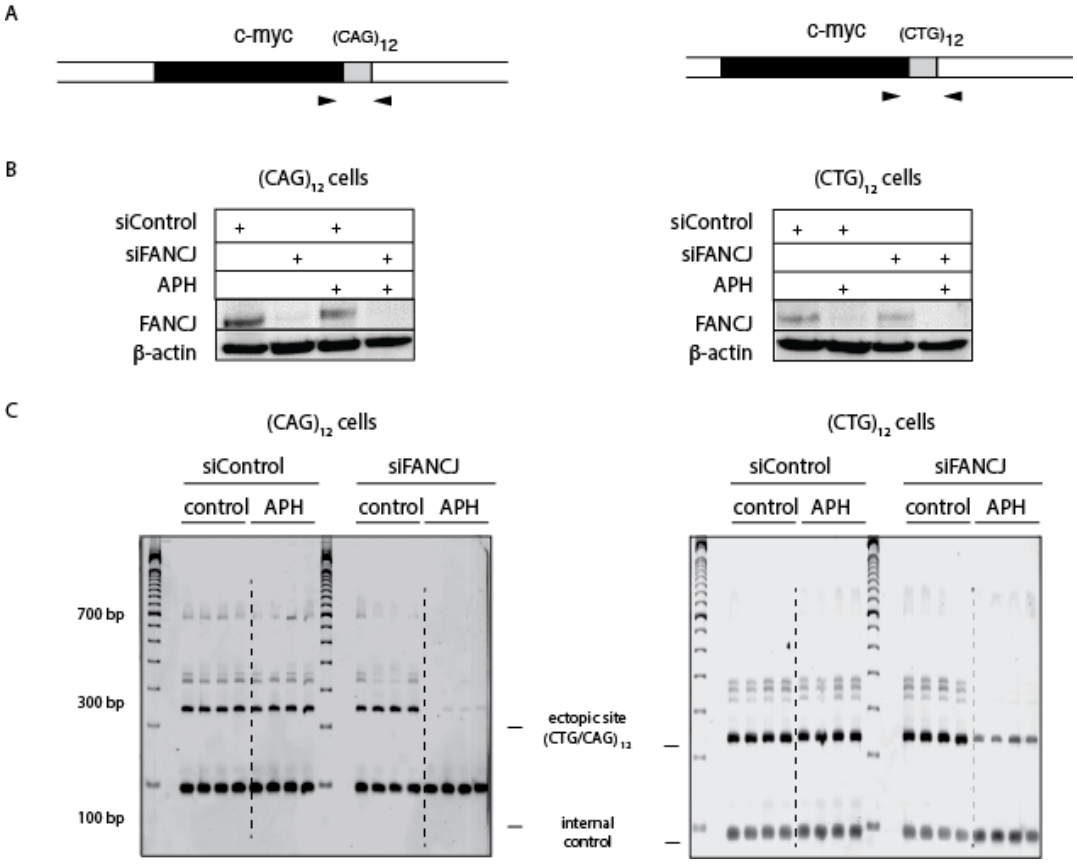

Figure 9. FANCJ knockdown leads to loss of ectopic (CTG)·(CAG) microsatellite signal in $(CTG)_{12}$·$(CAG)_{12}$ cells treated with aphidicolin. **(A)** Diagram of the integration site in $(CAG)_{12}$·$(CTG)_{12}$ cell lines. **(B)** Whole cell extracts were isolated after treatment of cells with siRNA against FANCJ and aphidicolin for a total of five transfections, or parallel untreated cultures, and Western blotted for FANCJ. **(C)** Small pool PCR results with primers flanking the ectopic $(CAG)_{12}$·$(CTG)_{12}$ repeats and primers for an internal control site.

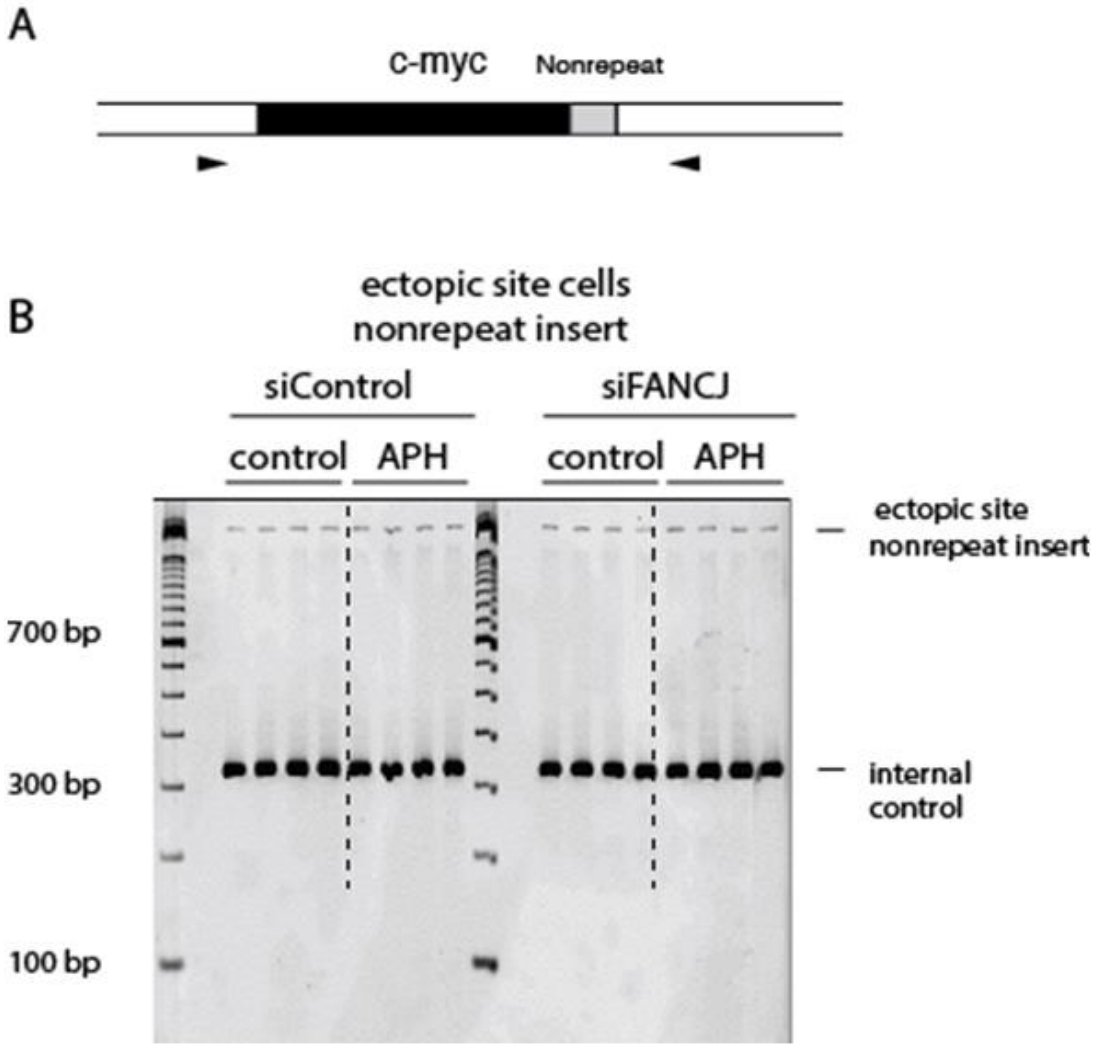

Figure 10. FANCJ knockdown does not lead to spPCR signal loss at a nonrepetitive sequence of the ectopic site. Small pool PCR results with primers flanking the nonrepetitive sequence at the ectopic site and primers for an internal control.

microsatellite instability is occurring due to the (CTG)·(CAG) repeats present at the ectopic site. Therefore, the ectopic site does not indiscriminately induce instability.

Small pool PCR is an end-point PCR method which can lead to an over estimate of a low abundance template compared to a higher abundant template that has plateaued amplification early in the PCR. To determine the sensitivity of our assay, we titrated CTG_{102} DNA, containing one ectopic site copy and four internal control site copies, with increasing amounts of HeLa DNA (four internal control site copies, no ectopic site copies) (Figure 11). A four-fold dilution of the ectopic copy by the internal control copy was easily detected (Figure 11). Therefore, we conclude the loss of PCR signal in cells treated with FANCJ knockdown and APH treatment represents the loss or separation of primer binding sites flanking the (CTG)·(CAG) repeats in a large majority (≥ 75%) of the cell population.

CTG_{102}·CAG_{102} cells were also treated with hydroxyurea (0.3 mM HU), a ribonucleotide reductase inhibitor, to deplete dNTP pools and inhibit DNA replication (Chen et al., 2015; Petermann et al., 2010). This low dose of HU slows replication forks and activates dormant origins to promote cell survival without initiating a DNA checkpoint response (Chen et al., 2015; Petermann et al., 2010). spPCR results revealed a loss of PCR signal at the ectopic site CTG_{102}·CAG_{102} repeats in cells that had been treated with FANCJ siRNA and HU (Figure 12). Taken together, these results suggest a role for FANCJ in the maintenance of (CTG)·(CAG) trinucleotide repeat stability during replicative stress (APH or HU) independent of (CTG)·(CAG) repeat replication polarity.

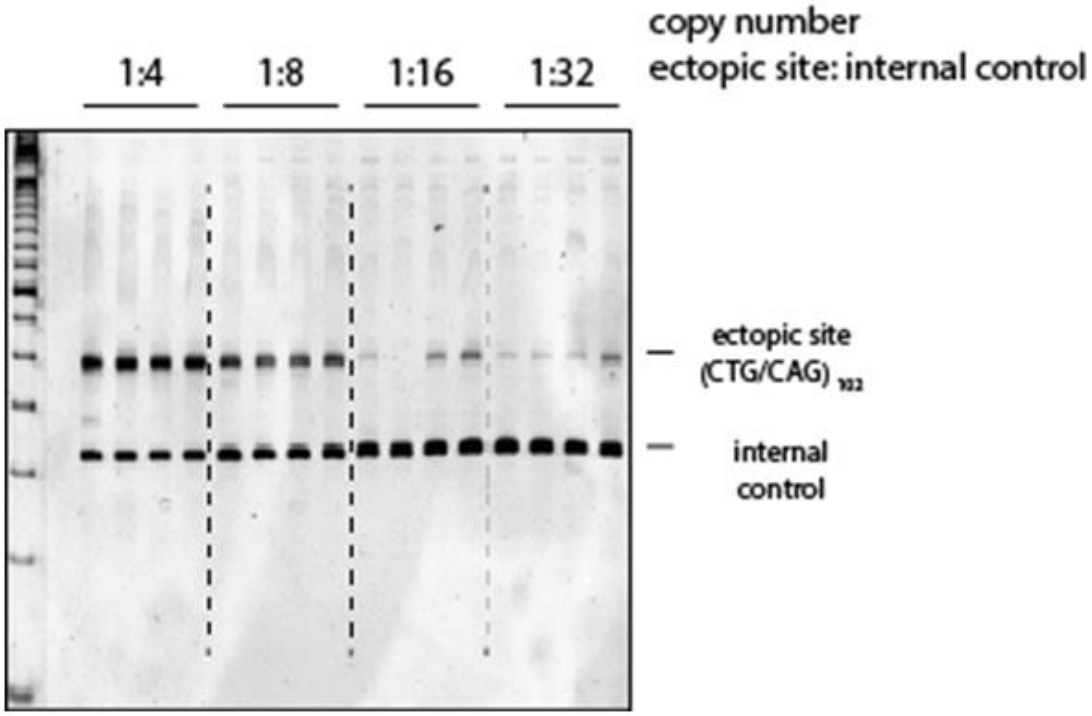

Figure 11. Titration of CTG_{102} cell DNA with HeLa cell DNA. Small pool PCR results of CTG_{102} cell DNA (1 ectopic site copy and 4 internal control site copies) was mixed with varying concentrations of HeLa DNA (4 internal control site copies and no ectopic site copies) resulting in template ratios of ectopic site: internal control of 1:4, 1:8, 1:16, 1:32.

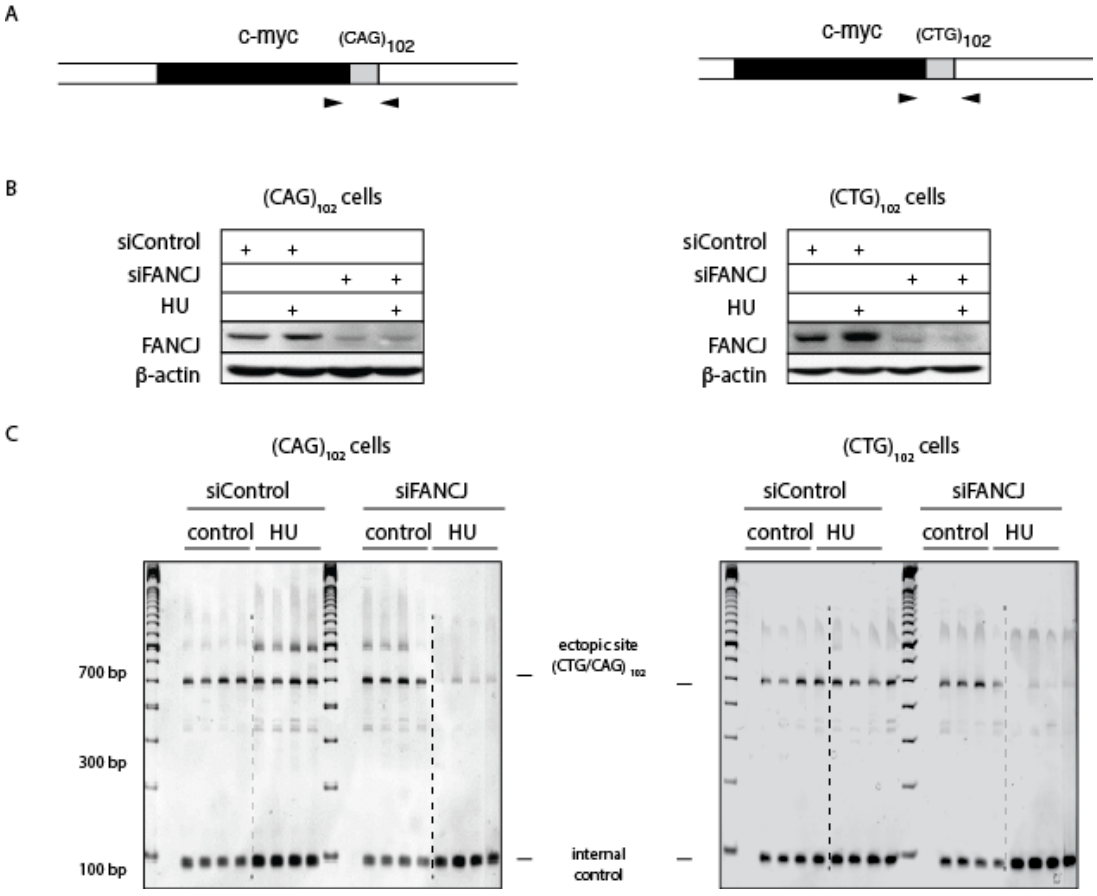

Figure 12. FANCJ knockdown leads to loss of ectopic (CTG)·(CAG) microsatellite signal in $(CTG)_{102}$·$(CAG)_{102}$ cells treated with hydroxyurea. **(A)** Diagram of the integration site in $(CAG)_{102}$·$(CTG)_{102}$ cell lines. **(B)** Whole cell extracts were isolated after treatment of cells with siRNA against FANCJ and hydroxyurea for a total of five transfections, or parallel untreated cultures, and immunoblotted for FANCJ. **(C)** Small pool PCR results with primers flanking the ectopic $(CAG)_{102}$·$(CTG)_{102}$ repeats and primers for a site without microsatellites serving as an internal control.

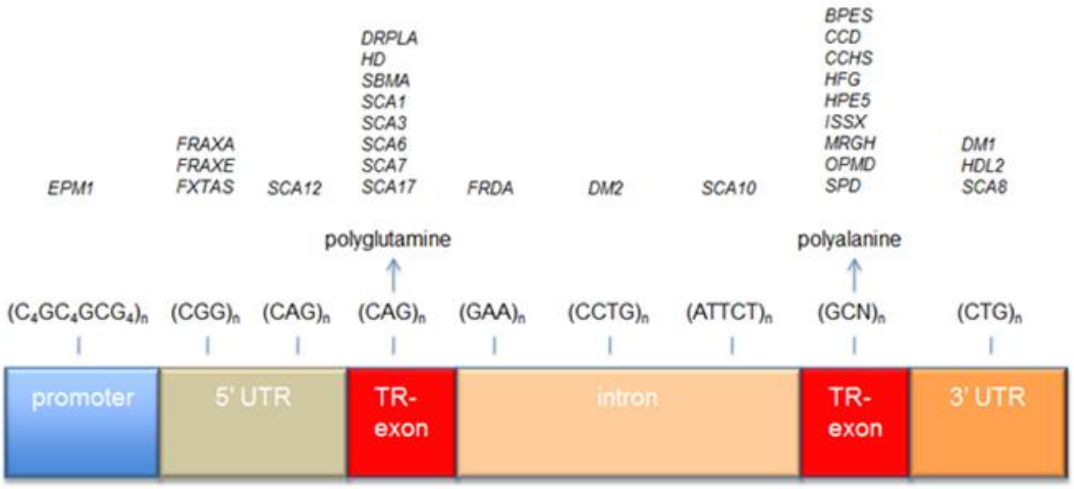

Figure 13: Expandable repeats associated with human disease. A schematic of microsatellites responsible for human disease and the corresponding location along the gene affected by the expansion of these repeated sequences.

FANCJ is necessary for endogenous microsatellite repeat stability

Trinucleotide repeats examined in preliminary experiments are only one class of microsatellites associated with disease (Collins et al., 2003). About 3% of the human genome consists of microsatellites, although not all of these microsatellites cause disease (Subramanian et al., 2003). Other types of disease associated microsatellites include tetra-nucleotide repeats, penta-nucleotide repeats, mirror repeats, and other trinucleotide repeated sequences (Figure 13) (Collins et al., 2003; McMurray, 2010; Subramanian et al., 2003). Since FANCJ works to protect the stability of (CTG)·(CAG) trinucleotide repeats; FANCJ may also have a function in the prevention of instability at other disease related microsatellites. To determine if this function of FANCJ is unique to (CTG)·(CAG) trinucleotide repeats, we examined multiple endogenous loci of different repeat composition. First, we confirmed (CTG)·(CAG) trinucleotide repeat stabilization by FANCJ with the analysis of the endogenous (CTG)·(CAG) repeats of the dystrophia myotonica protein kinase (DMPK, chr 19q13.32) gene, spinocerebellar ataxia type 1 (SCA1, chr 6p22.3) gene, transcription factor 4 (TCF4, chr 18q21.2) gene (referred to also as CTG18.1), and expanded repeat domain 1 (ERDA1, chr q21.3) loci. As observed for the ectopic site (CTG)·(CAG) repeat, the spPCR results from CAG_{102} cells depleted of FANCJ and treated with APH showed PCR signal loss at these endogenous loci (Figure 14). Next, we examined the effect of loss of FANCJ during replicative stress (APH) on other non-(CTG)·(CAG) disease associated loci including the penta-nucleotide ATTCT repeat of the spinocerebellar ataxia type 10 (SCA10, chr22q13.31) locus, and the polypurine·polypyrimidine (Pu·Py) repeat track (thought to form triplexes and/or G-quadruplexes) of the polycystic kidney disease 1 (PKD1, chr 16p13.3, autosomal

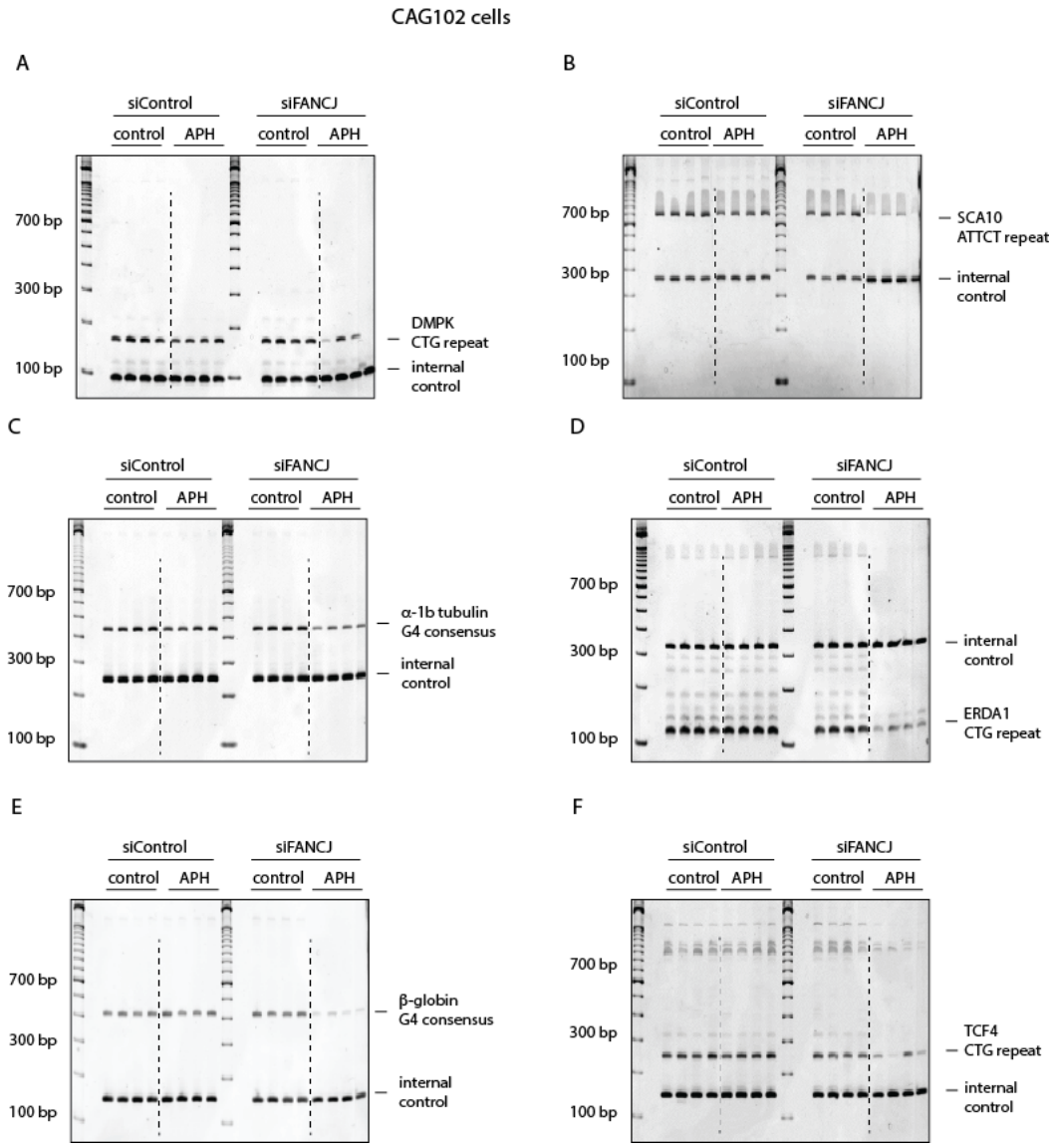

Figure 14. FANCJ knockdown leads to the loss PCR signal at multiple endogenous microsatellites with aphidicolin treatment. Small pool PCR results from endogenous DMPK (CTG repeat), SCA10 (ATTCT repeat), PKD1 (Pu·Py mirror repeat), α-1B tubulin (G4 DNA, G-quadruplex), ERDA1 (CTG repeat), SCA1 (CTG repeat), β-globin (G4 DNA), TCF4 (CTG repeat), FANCJ/BRIP1 (polyT repeat), c-MYC (G4 DNA), p53 (poly T repeat), and p63 (poly T repeat) in cells treated with siFANCJ or siControl and aphidicolin.

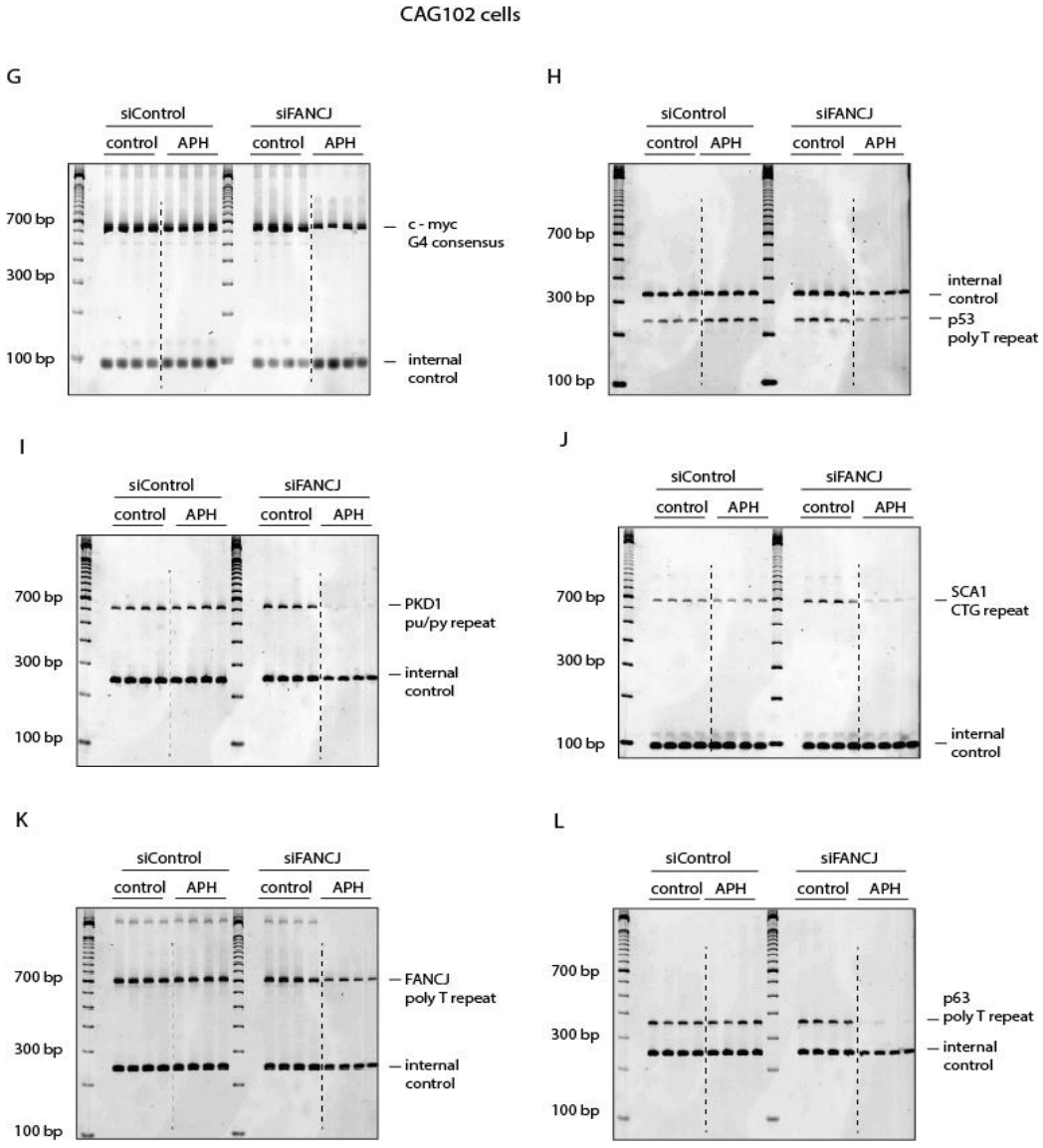

Figure 14 (continued). FANCJ knockdown leads to the loss PCR signal at multiple endogenous microsatellites with aphidicolin treatment. Small pool PCR results from endogenous DMPK (CTG repeat), SCA10 (ATTCT repeat), PKD1 (Pu·Py mirror repeat), α-1B tubulin (G4 DNA, G-quadruplex), ERDA1 (CTG repeat), SCA1 (CTG repeat), β-globin (G4 DNA), TCF4 (CTG repeat), FANCJ/BRIP1 (polyT repeat), c-MYC (G4 DNA), p53 (poly T repeat), and p63 (poly T repeat) in cells treated with siFANCJ or siControl and aphidicolin.

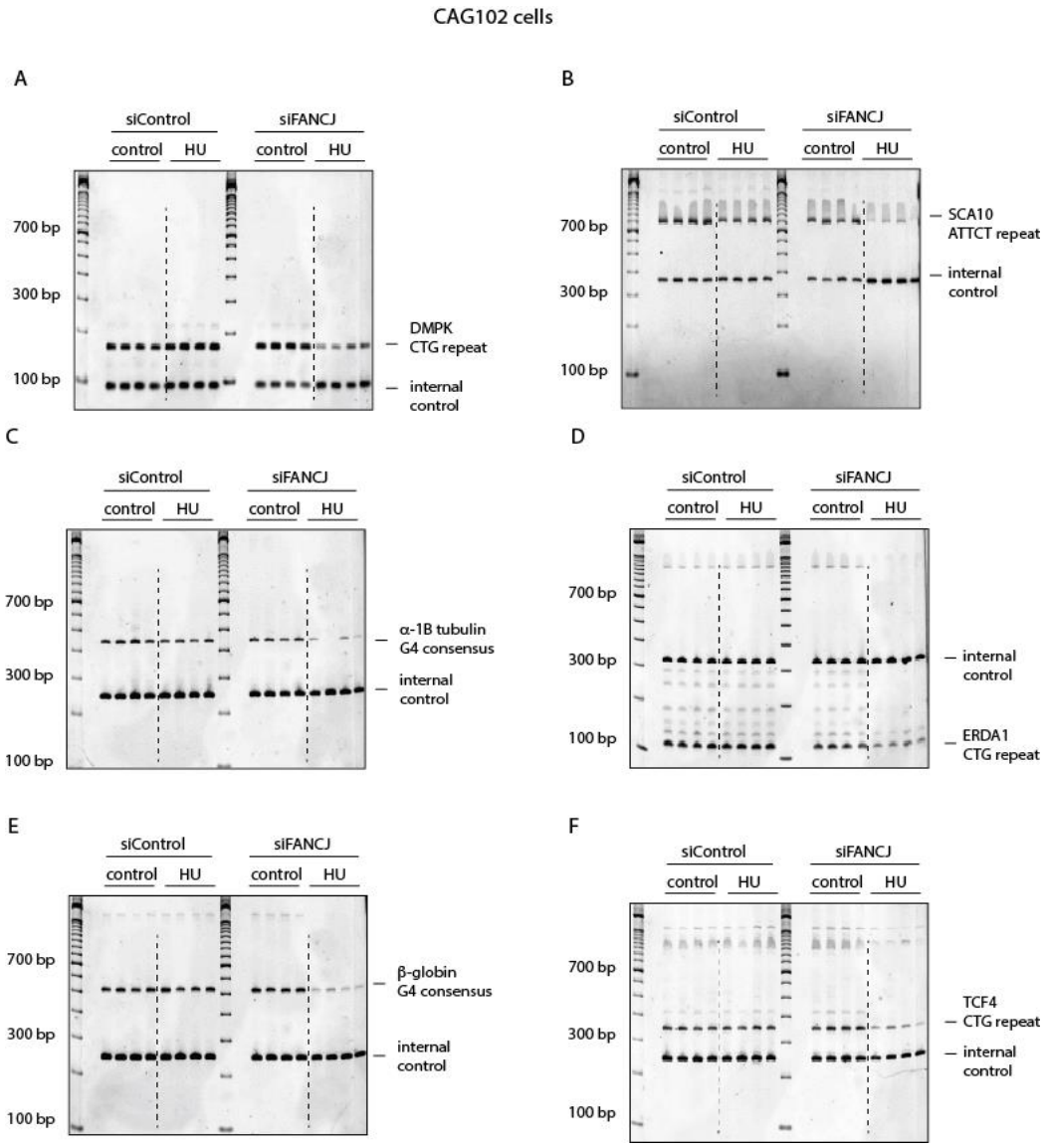

Figure 15. FANCJ knockdown leads to the loss PCR signal at multiple endogenous microsatellites with hydroxyurea treatment. Small pool PCR results from endogenous DMPK (CTG repeat), SCA10 (ATTCT repeat), PKD1 (Pu·Py mirror repeat), α-1B tubulin (G4 DNA, G-quadruplex), ERDA1 (CTG repeat), SCA1 (CTG repeat), β-globin (G4 DNA), TCF4 (CTG repeat), FANCJ/BRIP1 (polyT repeat), c-MYC (G4 DNA), p53 (poly T repeat), and p63 (poly T repeat) in cells treated with siFANCJ or siControl and hydroxyurea.

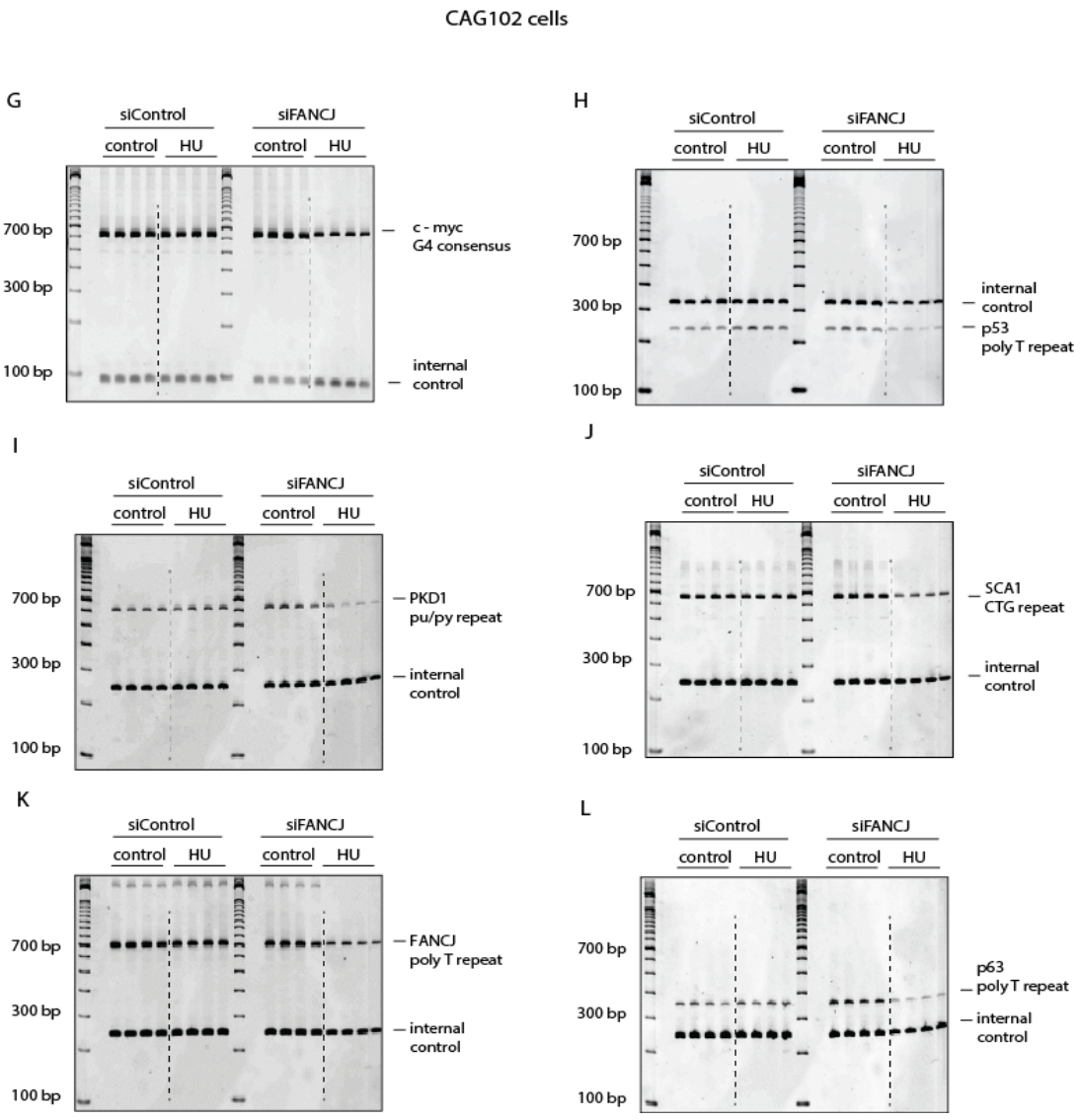

Figure 15 (continued). FANCJ knockdown leads to the loss PCR signal at multiple endogenous microsatellites with hydroxyurea treatment. Small pool PCR results from endogenous DMPK (CTG repeat), SCA10 (ATTCT repeat), PKD1 (Pu·Py mirror repeat), α-1B tubulin (G4 DNA, G-quadruplex), ERDA1 (CTG repeat), SCA1 (CTG repeat), β-globin (G4 DNA), TCF4 (CTG repeat), FANCJ/BRIP1 (polyT repeat), c-MYC (G4 DNA), p53 (poly T repeat), and p63 (poly T repeat) in cells treated with siFANCJ or siControl and hydroxyurea.

dominant polycystic kidney disease). These additional disease associated microsatellite sequences examined vary in nucleotide composition, length, and location throughout the genome. Treatment of CAG_{102} cells with FANCJ siRNA and APH lead to spPCR results displaying a loss of PCR signal at all endogenous microsatellites analyzed (Figure 14). Additionally, we examined repeat sequences which are not associated with a microsatellite disorder including a mono-nucleotide poly T repeat in p63 (chr 3q28), FANCJ/BRIP1 (17q23.2), and p53 (chr 17p13.1), as well as G4 DNA (known to form G-quadruplexes) of β-globin (chr 11p15.4), α-1B tubulin (chr 12q13.12), and c-MYC (chr 8q24.21). In the absence of FANCJ and presence of APH, a loss of PCR signal was observed in CAG_{102} cells. Similar results were obtained from spPCR results of CAG_{102} cells depleted of FANCJ and treated with HU, in which all endogenous microsatellites examined showed a loss of PCR signal (Figure 15). Since the loss of PCR signal is not specific to (CTG)·(CAG) repeat tracks, we conclude FANCJ is essential for the stabilization of microsatellites across the genome during replicative stress irrespective of nucleotide composition and length.

To account for possible off-target effects of siRNA treatment, immortalized Fanconi anemia complementation group J patient fibroblasts (FANCJ null) harboring a biallelic frameshift mutation (c.308delG, p.F103fs) were treated with aphidicolin (0.5 μM APH). spPCR analysis of all the endogenous microsatellites previously tested revealed a dramatic loss of PCR signal compared to internal controls (Figure 16). FANCJ null cells treated with HU also displayed PCR signal loss (Figure 17) further strengthening our finding that replication stress causes microsatellite instability in FANCJ null or depleted cells during replication stress.

While performing APH treatment of FANCJ knocked down and null cells, we noticed a hypersensitivity to the APH compared to control cells. We counted CAG_{102} cells treated with FANCJ or control siRNA in APH as well as FANCJ null, wild-type and FANCA null fibroblast cells treated with APH and confirmed a decrease in the relative cell number of FANCJ knocked down or null cells (Figure 18).

Next we sought to determine if the phenotype of PCR signal loss at endogenous microsatellite sequences could be rescued with the expression of FANCJ wild-type cDNA. As expected, expression of wild-type FANCJ cDNA (Figure 19) in FANCJ null cells treated with APH (Figure 20) or HU (Figure 21) recovered spPCR signal loss at all tested endogenous microsatellite sequences.

Although the dose of aphidicolin used in these experiments has previously been shown to slow replication fork progress without activating the DNA checkpoint response, the spPCR signal loss seems too dramatic to remain under the detection of the checkpoint response. Therefore, we examined the cell cycle profiles of FANCJ null cells treated with 0.5 μM APH at different time points using the DNA stain propidium iodide and flow cytometry. Starting as early as 2 days of treatment with APH, FANCJ null cells begin to arrest in G2/M when compared to untreated control cells (Figure 22). Coupled with the robust loss of PCR signaling at microsatellites, we conclude that double strand breaks occur at microsatellites eventually causing a DNA checkpoint response and delayed cell division.

Summary

The presented data suggest a novel role for the DNA helicase FANCJ in microsatellite stabilization across the genome during replicative stress. To begin we

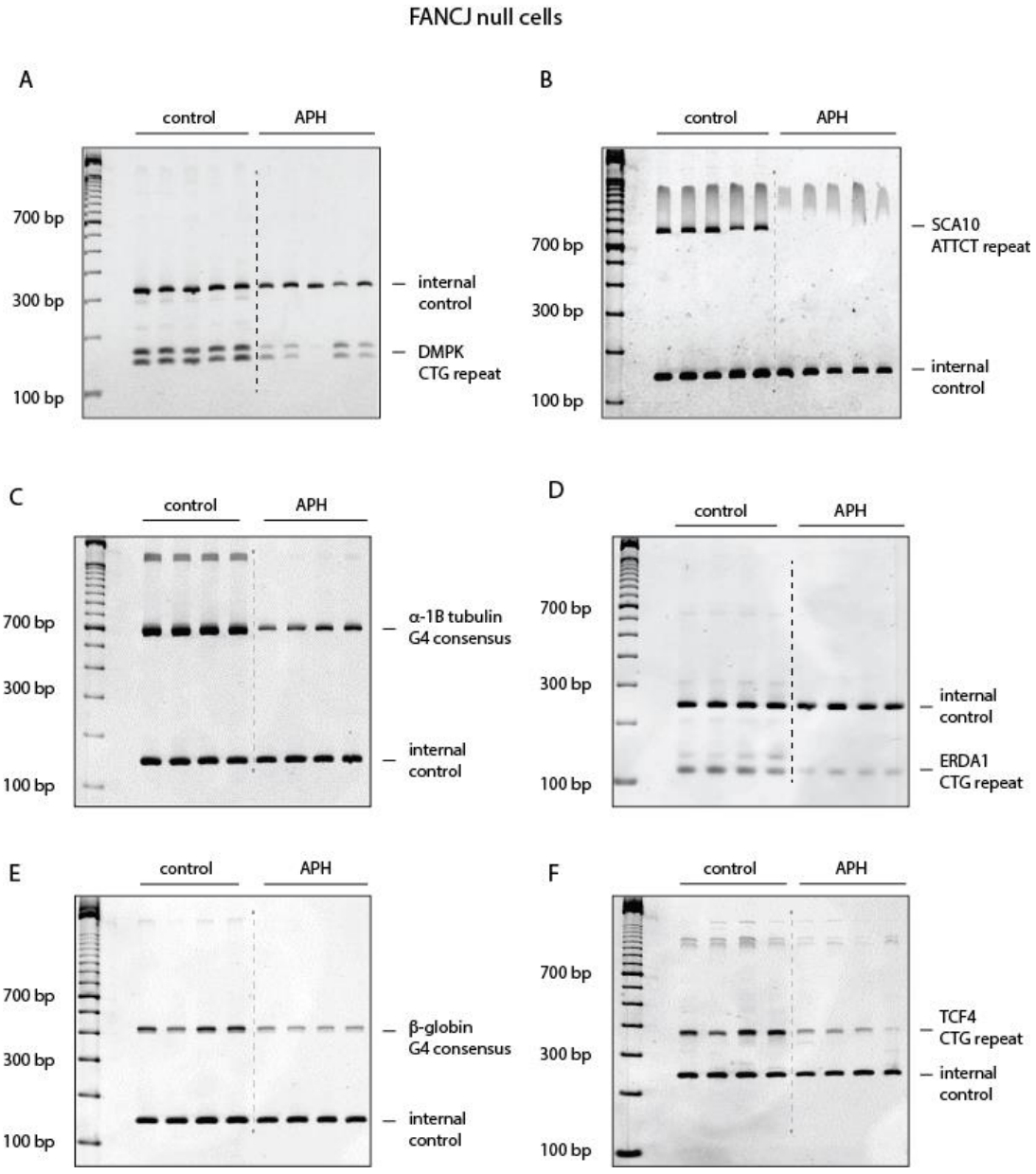

Figure 16. FANCJ null cells show loss of microsatellite signals after treatment with aphidicolin. Small pool PCR across endogenous microsatellite sites using DNA from FANCJ null cells treated with aphidicolin.

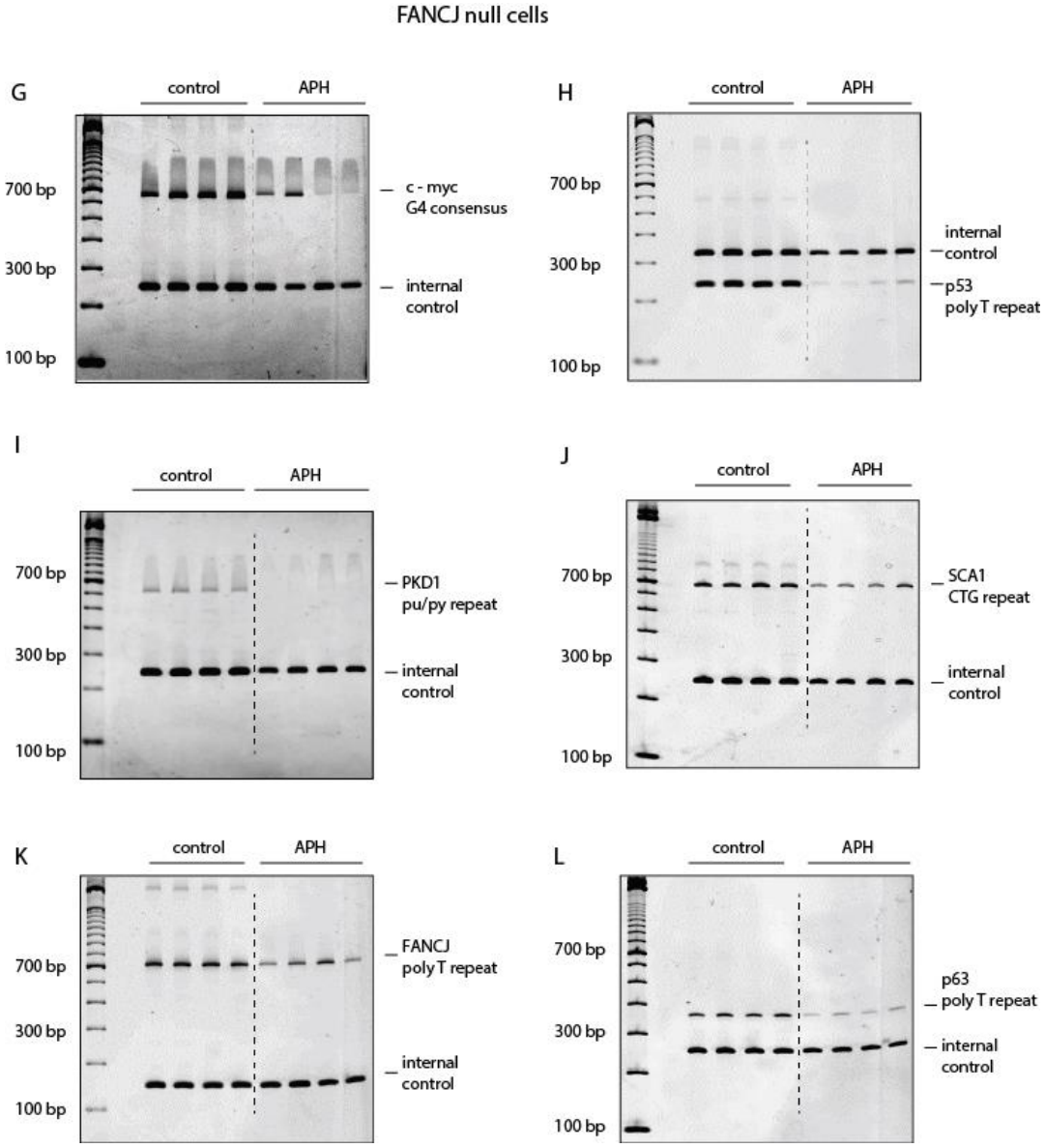

Figure 16 (continued). FANCJ null cells show loss of microsatellite signals after treatment with aphidicolin. Small pool PCR across endogenous microsatellite sites using DNA from FANCJ null cells treated with aphidicolin.

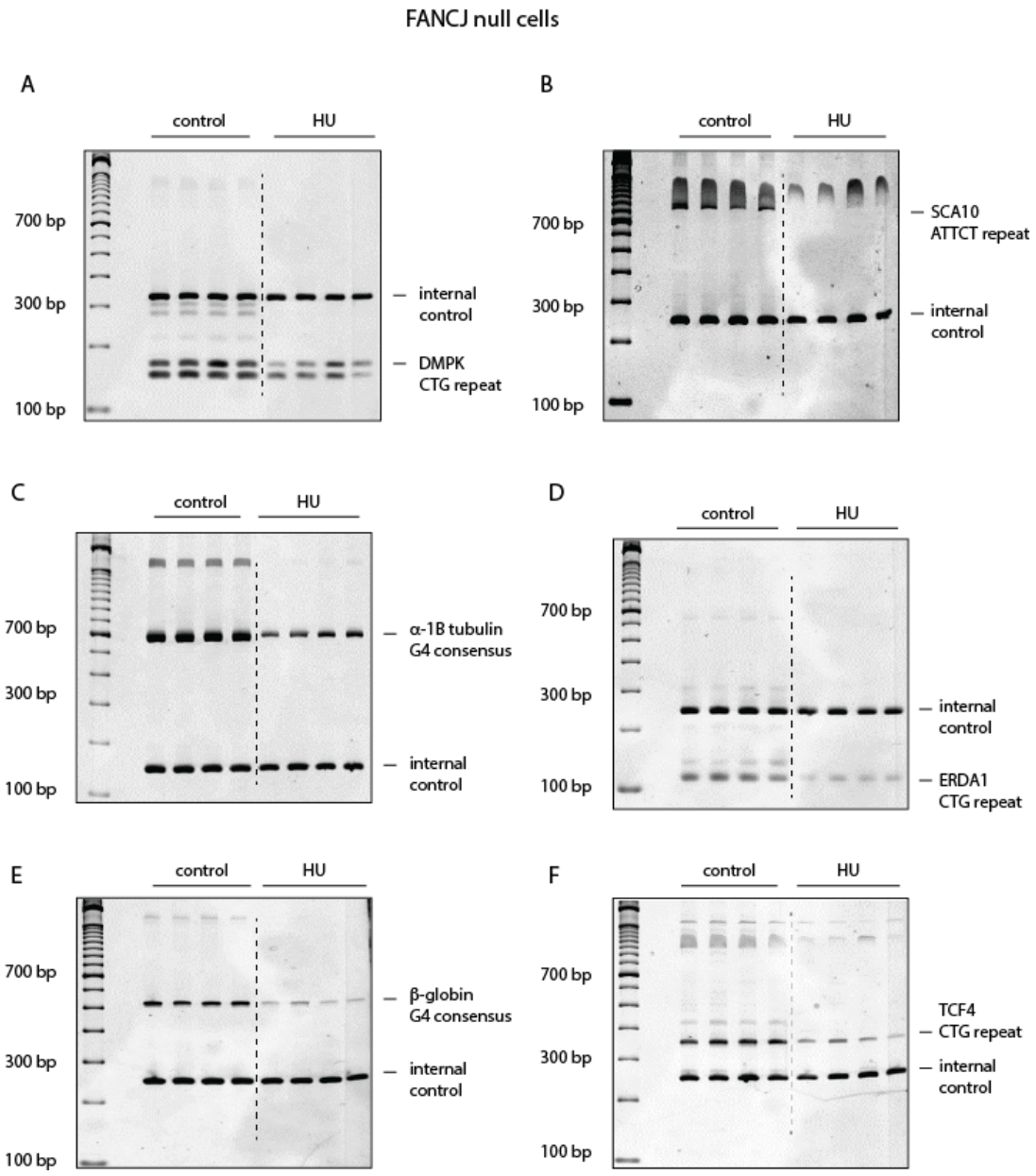

Figure 17. FANCJ null cells show loss of microsatellite signals after treatment with hydroxyurea. Small pool PCR across endogenous microsatellite sites using DNA from FANCJ null cells treated with hydroxyurea.

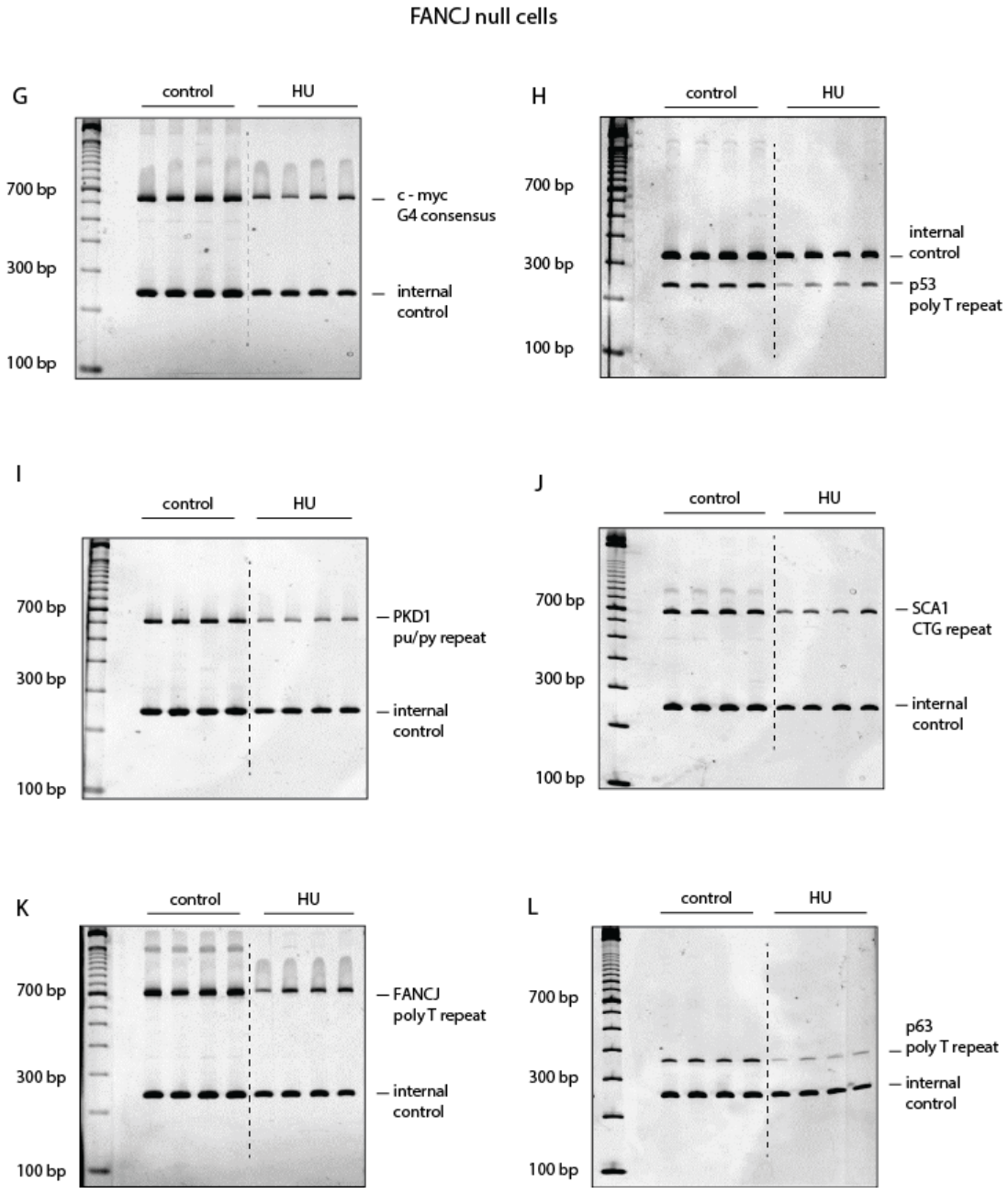

Figure 17 (continued). FANCJ null cells show loss of microsatellite signals after treatment with hydroxyurea. Small pool PCR across endogenous microsatellite sites using DNA from FANCJ null cells treated with hydroxyurea.

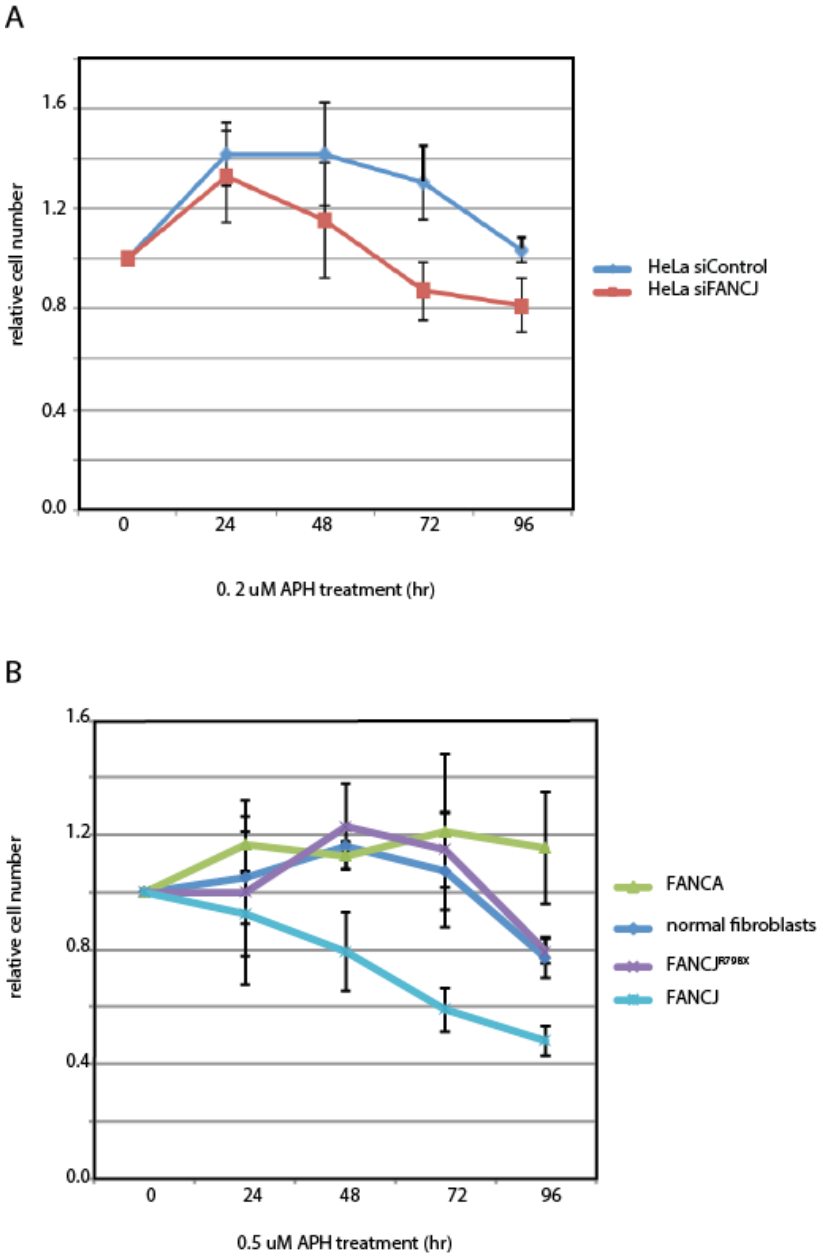

Figure 18. FANCJ depleted cells are hypersensitive to aphidicolin. (A) CAG_{102} cells treated with control of FANCJ siRNA and aphidicolin for 96 hours and counted every 24 hours. **(B)** FANCJ null, FANCA null, $FANCJ^{R798X}$, and wild-type fibroblast cells treated with aphidicolin continuously for 96 hours and counted every 24 hours.

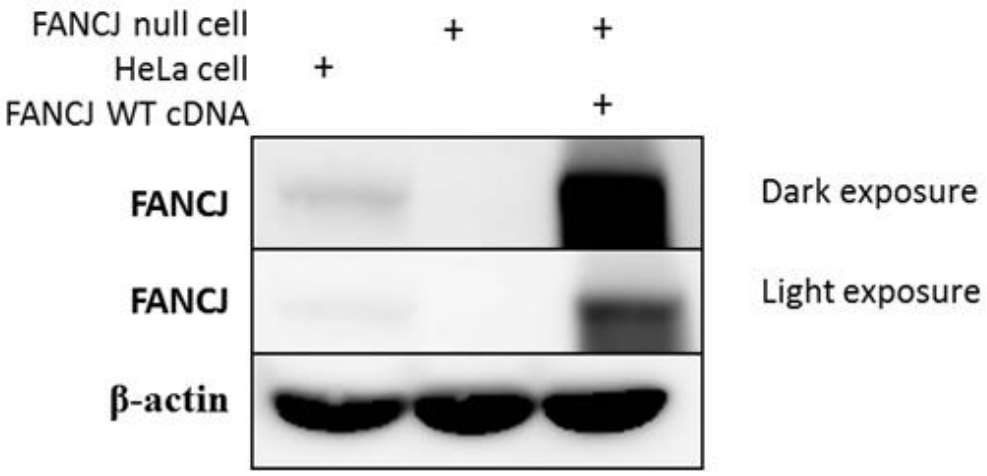

Figure 19. Expression of FANCJ wild-type cDNA in FANCJ null cells. Whole cell lysates of FANCJ null, FANCJ null complimented with FANCJ wild-type cDNA, and HeLa cells probed for FANCJ protein.

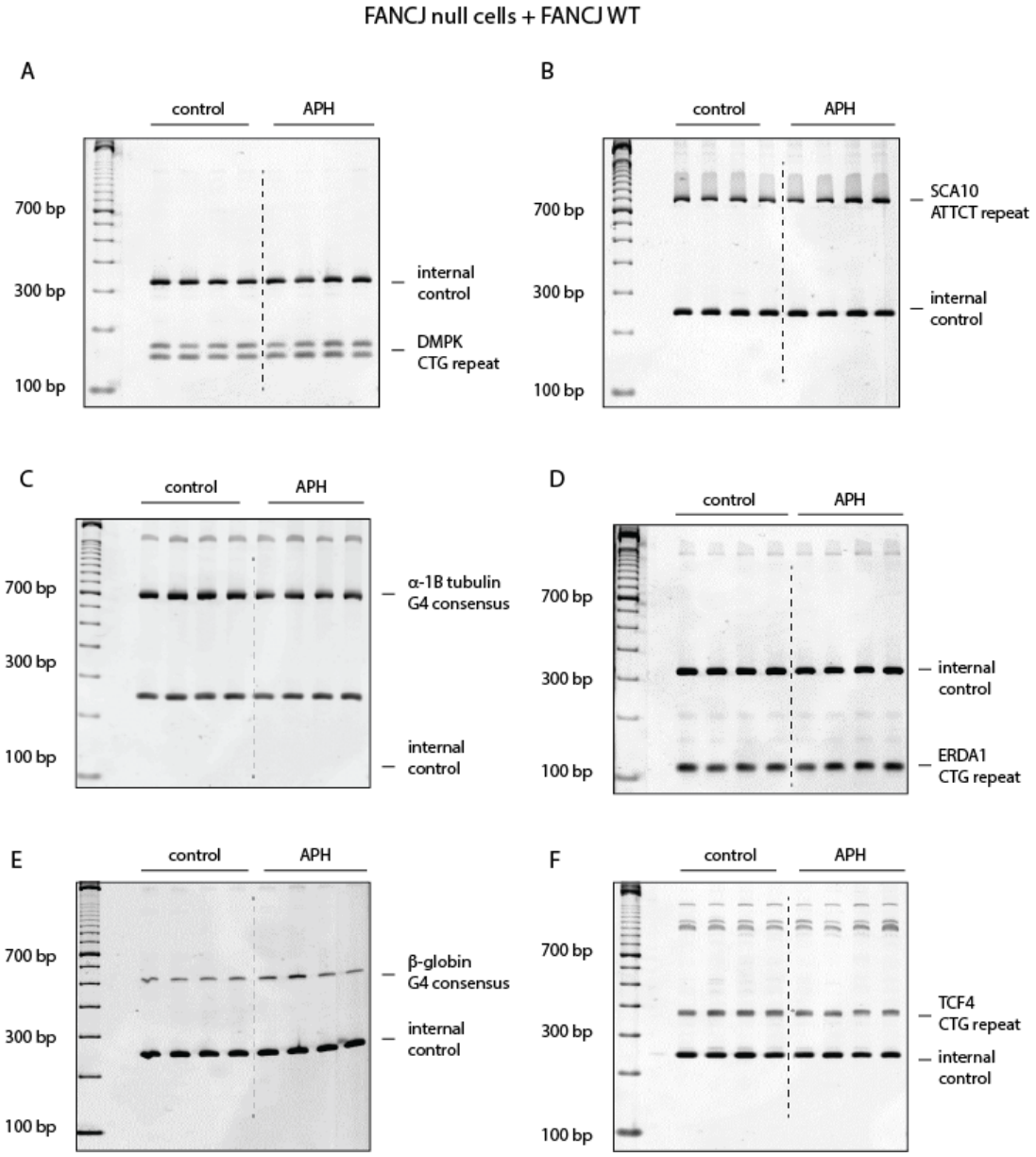

Figure 20. Aphidicolin treatment of FANCJ null +FANCJ WT cDNA cells. Small pool PCR results for all endogenous microsatellites in FANCJ null cells complimented with FANCJ wild-type cDNA treated with aphidicolin.

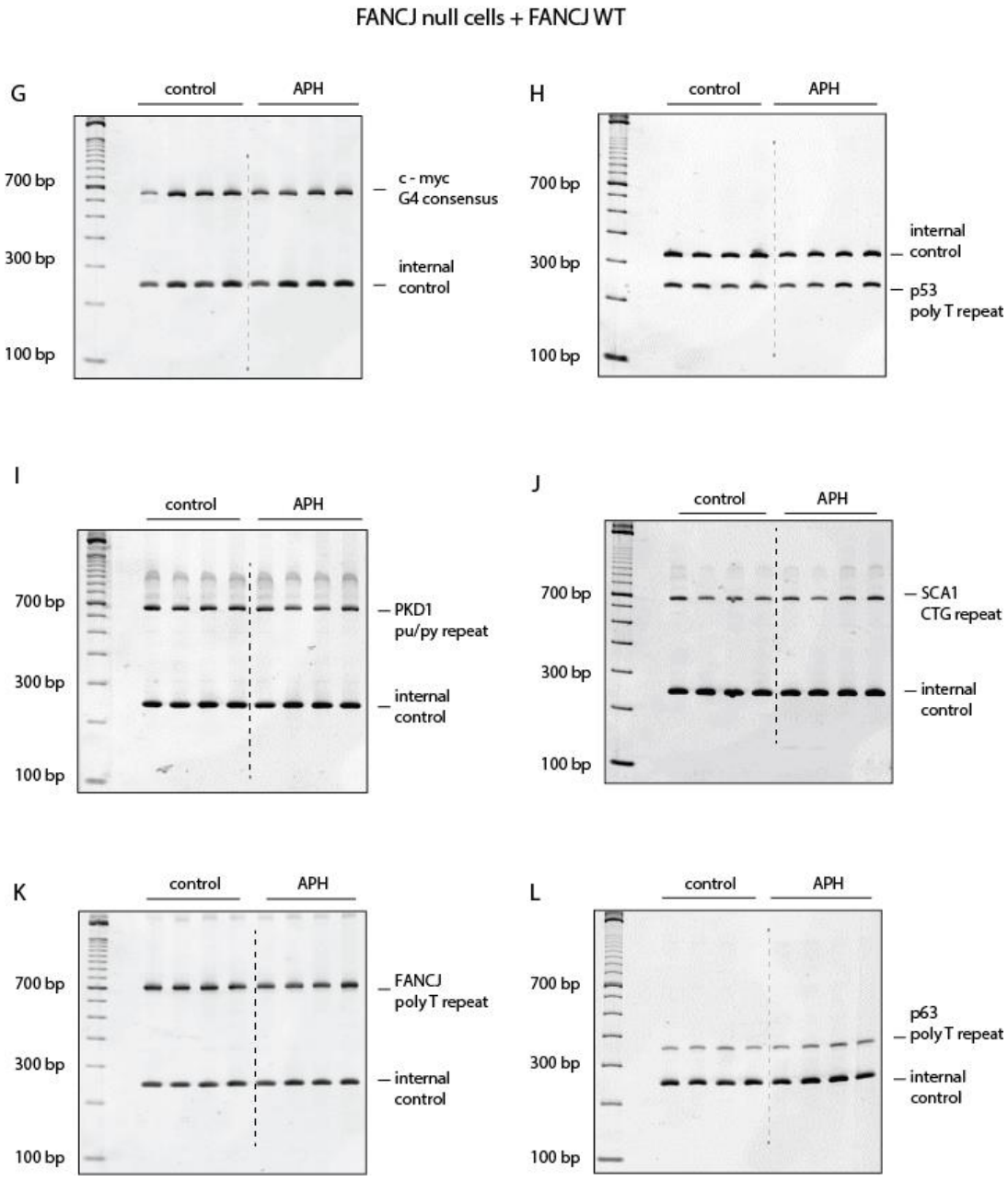

Figure 20 (continued). Aphidicolin treatment of FANCJ null +FANCJ WT cDNA cells. Small pool PCR results for all endogenous microsatellites in FANCJ null cells complimented with FANCJ wild-type cDNA treated with aphidicolin.

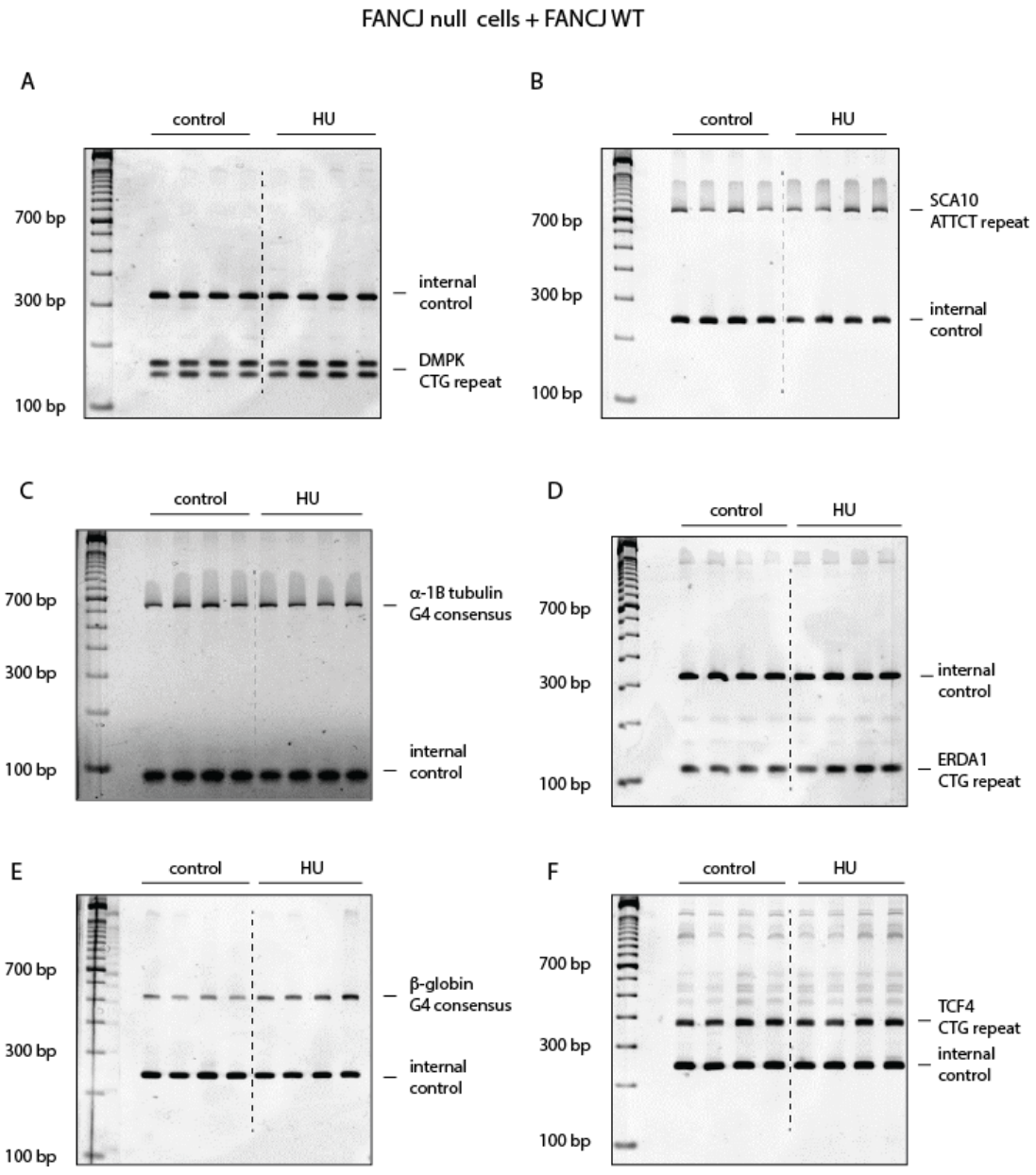

Figure 21. Hydroxyurea treatment of FANCJ null +FANCJ WT cDNA cells. Small pool PCR results for all endogenous microsatellites in FANCJ null cells complimented with FANCJ wild-type cDNA treated with hydroxyurea.

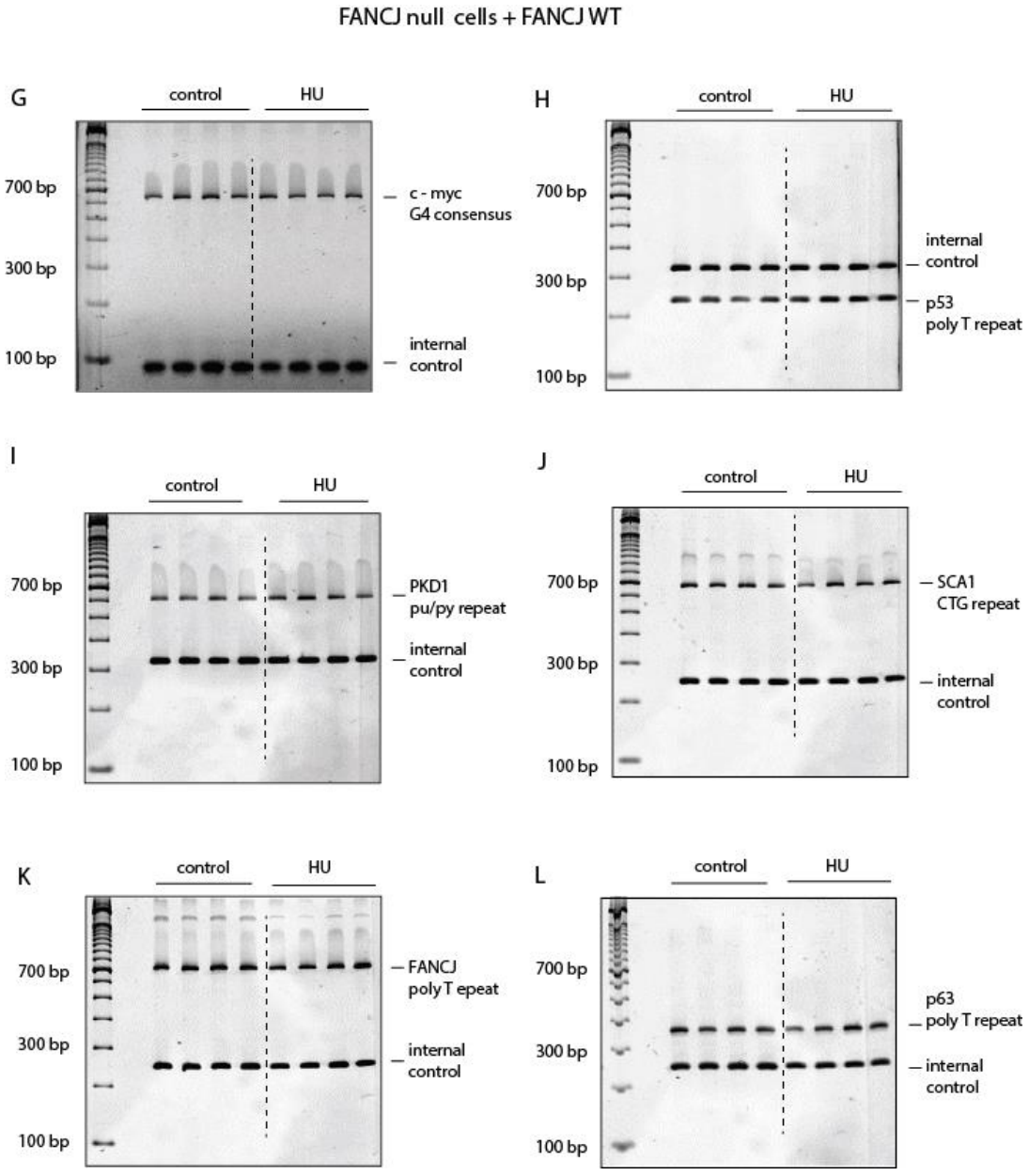

Figure 21 (continued). Hydroxyurea treatment of FANCJ null +FANCJ WT cDNA cells. Small pool PCR results for all endogenous microsatellites in FANCJ null cells complimented with FANCJ wild-type cDNA treated with hydroxyurea.

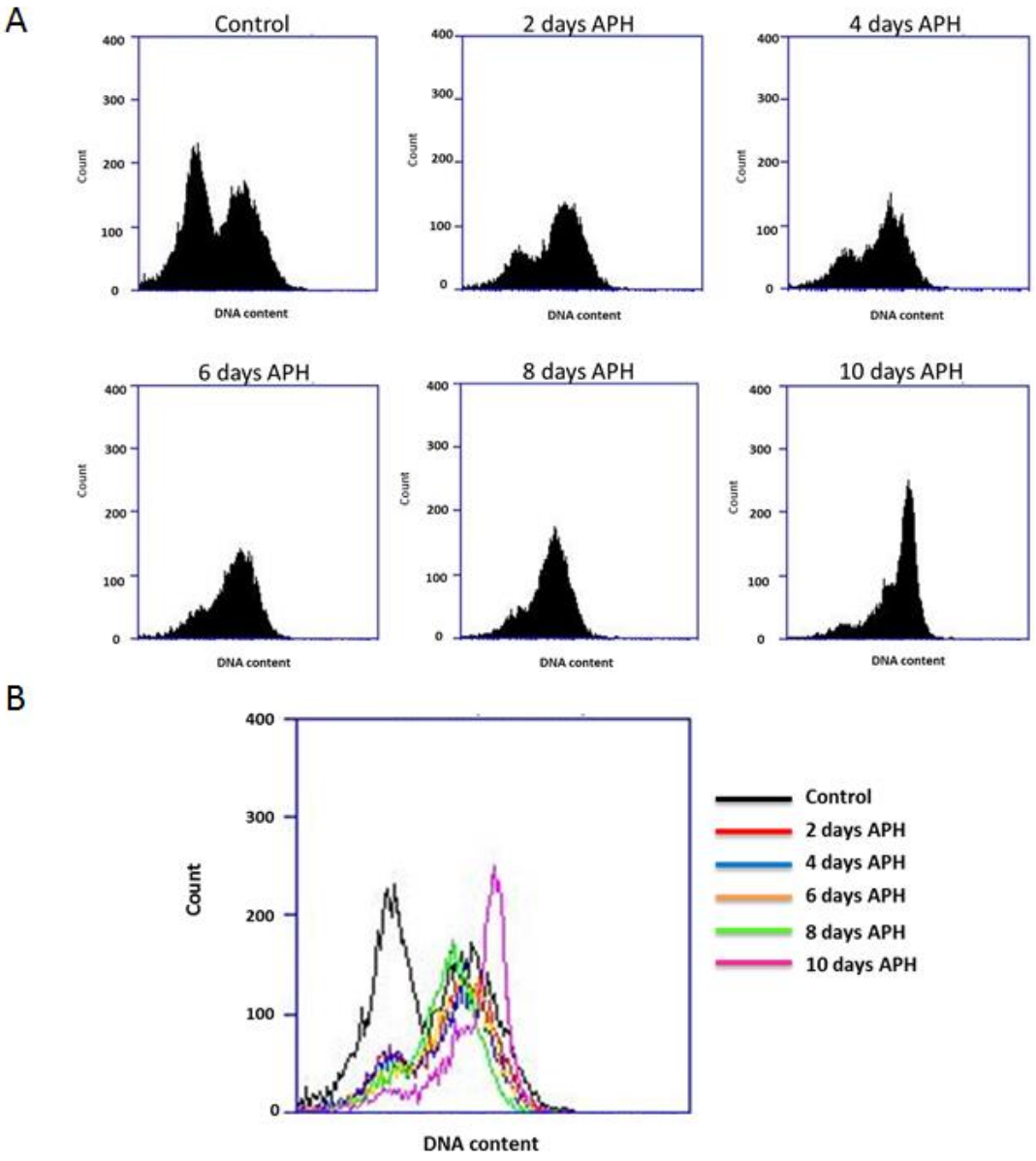

Figure 22. Cell cycle profiles of FANCJ null cells treated with aphidicolin. (A) Propidium iodide DNA stained FANCJ null cells analyzed by flow cytometry after 2, 4, 6, 8, or 10 days of aphidicolin treatment. **(B)** Composite of cell cycle profiles.

showed (CTG)·(CAG) trinucleotide repeat instability at an ectopic site integrated into the HeLa genome in the absence of FANCJ and presence of either APH or HU. However, as shown by evaluation of multiple endogenous microsatellite sequences in CAG_{102} cells treated with FANCJ siRNA and APH or HU by spPCR, the function of FANCJ in microsatellite stabilization has broad effects. These results were confirmed with the treatment of FANCJ null cells with APH or HU. Lastly, expression of wild-type FANCJ cDNA in FANCJ null cells rescued the effects of APH or HU treatment establishing a direct relationship between FANCJ and microsatellite instability during replicative stress.

II. FANCJ functions independently of the FA repair pathway

FA repair pathway activity is not necessary for microsatellite stabilization by FANCJ

The helicase FANCJ plays a role in the replication dependent Fanconi anemia (FA) repair pathway (Kee and D'Andrea, 2010). Classical models of the FA repair pathway imply a function to resolve interstrand crosslinks (Kee and D'Andrea, 2010). However, recent literature suggests a broader role for the FA repair pathway in the resolution of other types of DNA damage including double strand breaks (DSBs) (Kee and D'Andrea, 2010). The presence of an interstrand crosslink within DNA activates a signaling cascade beginning with the phosphorylation of FANCM followed by the assembly of a core complex, consisting 9 other FANC proteins (Kee and D'Andrea, 2010). Once the core complex assembles, the E3 ubiquitin ligase, FANCL, becomes active and monoubiquitinates both FANCD2 and FANCI leading to heterodimer formation of FANCI and FANCD2 and localization of both proteins at the site of the

interstrand crosslink (Garner and Smogorzewska, 2011; Kee and D'Andrea, 2010). Subsequently, downstream effectors, including the helicase FANCJ, localize to the damaged DNA for repair to occur (Kee and D'Andrea, 2010). The activation of the FA repair pathway is depicted in Figure 3. Since the loss of FANCJ coupled with aphidicolin treatment causes instability at a variety of microsatellite sequences producing double strand breaks, FANCJ may work in conjunction with the entire FA pathway to play a role in genome stabilization. To determine if the activation of the FA repair pathway is required for FANCJ to maintain microsatellite stability, we first knocked down the initiator protein of the pathway, FANCM, with a pool of siRNAs in $CTG_{102}{\cdot}CAG_{102}$ cells with and without APH (Figure 23). Results for spPCR experiments using primers flanking the ectopic site (CTG)·(CAG) repeats revealed no loss of PCR signal in contrast to cells treated with siFANCJ and APH. In addition, all of the endogenous microsatellites previously examined also showed no loss of PCR signal in the absence of FANCM and presence of APH (Figure 24) suggesting FANCJ functions independent of FANCM phosphorylation to maintain microsatellite stability.

To determine the requirement of FA core complex assembly on FANCJ stabilized microsatellites, immortalized FANCA, FANCC, and FANCL null patient cells lines were obtained. FA core complex cell lines were treated with either APH or HU for 10 days as were immortalized wild-type fibroblast cells as a control. FANCA (Figure 25 and 26), FANCC (Figure 27 and 28), FANCL (Figure 29 and 30) null APH or HU treated cells produced spPCR products in which no loss of PCR signal was observed at any endogenous microsatellite analyzed. Likewise, no microsatellite instability was observed in wild-type fibroblast cells treated with aphidicolin (Figure 31) or hydroxyurea (Figure

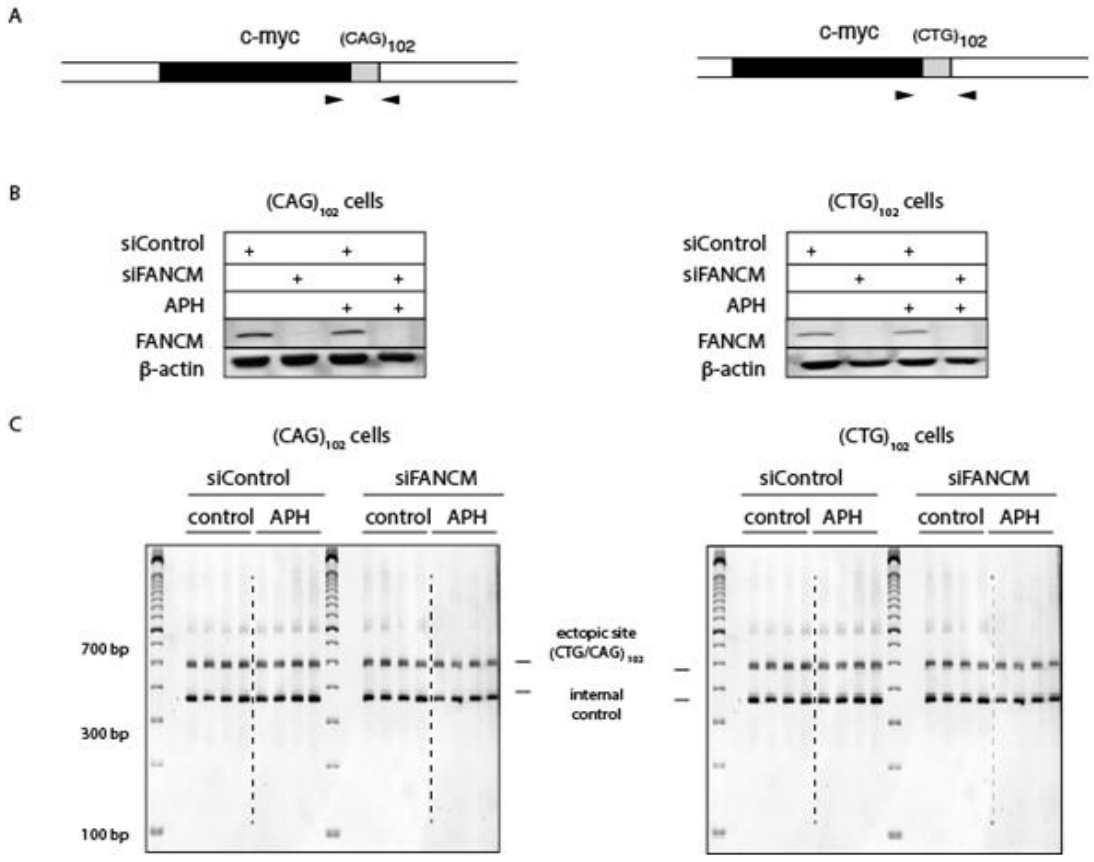

Figure 23. FANCM knockdown does not lead to loss of ectopic (CTG)·(CAG) microsatellite signal in $(CTG)_{102}$·$(CAG)_{102}$ cells treated with aphidicolin. **(A)** Diagram of the integration site in $(CAG)_{102}$·$(CTG)_{102}$ cell lines. **(B)** Whole cell extracts were isolated after treatment of cells with siRNA against FANCM and aphidicolin for a total of five transfections, or parallel untreated cultures, and immunoblotted for FANCM. **(C)** Small pool PCR results with primers flanking the ectopic $(CAG)_{102}$·$(CTG)_{102}$ repeats and primers for a site without microsatellites serving as an internal control.

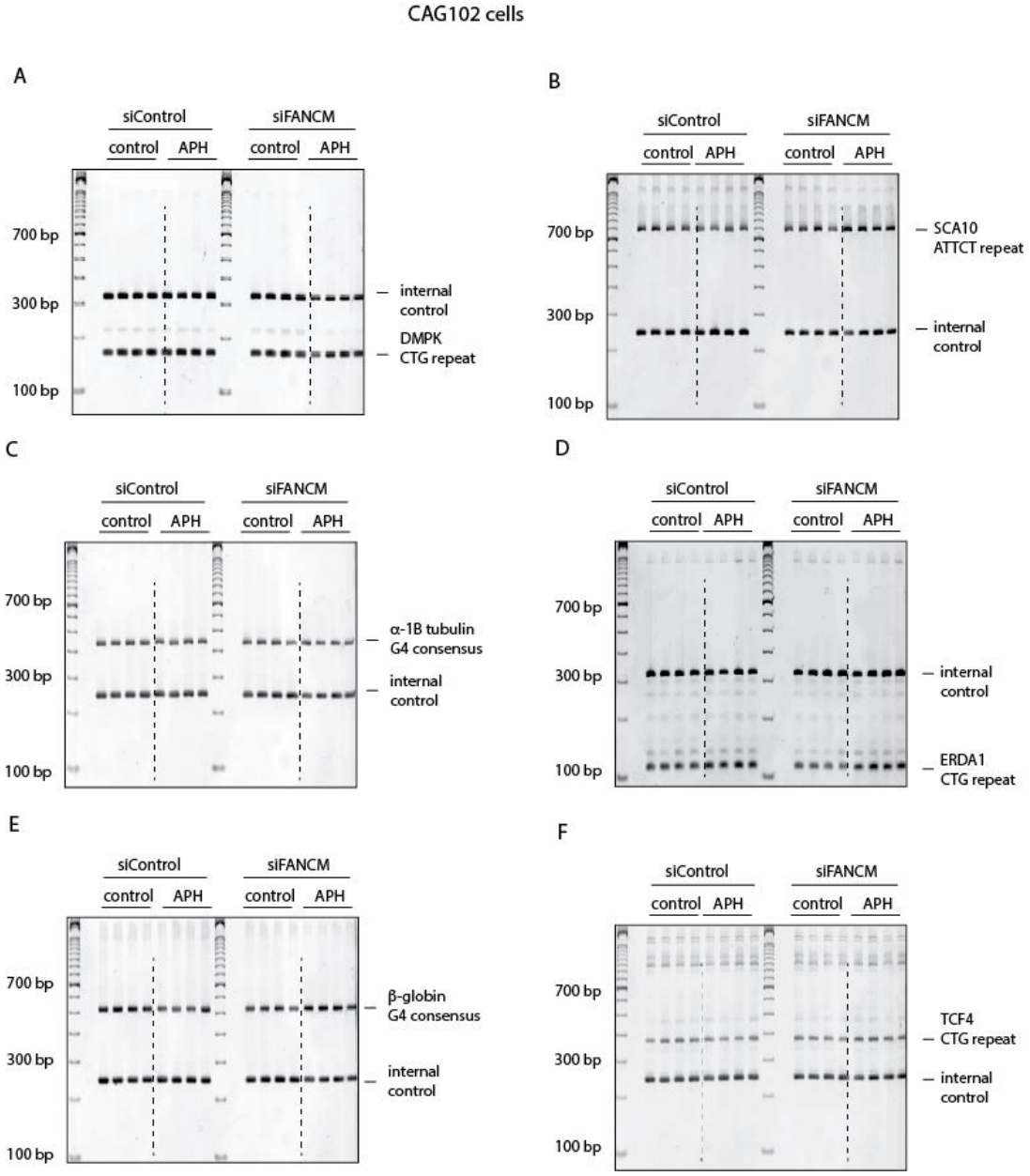

Figure 24. FANCM knockdown does not lead to loss of PCR signal at multiple endogenous sites with aphidicolin treatment. Small pool PCR results of CAG_{102} cells treated with siFANCM and aphidicolin. No loss of PCR signal was observed at multiple endogenous microsatellites.

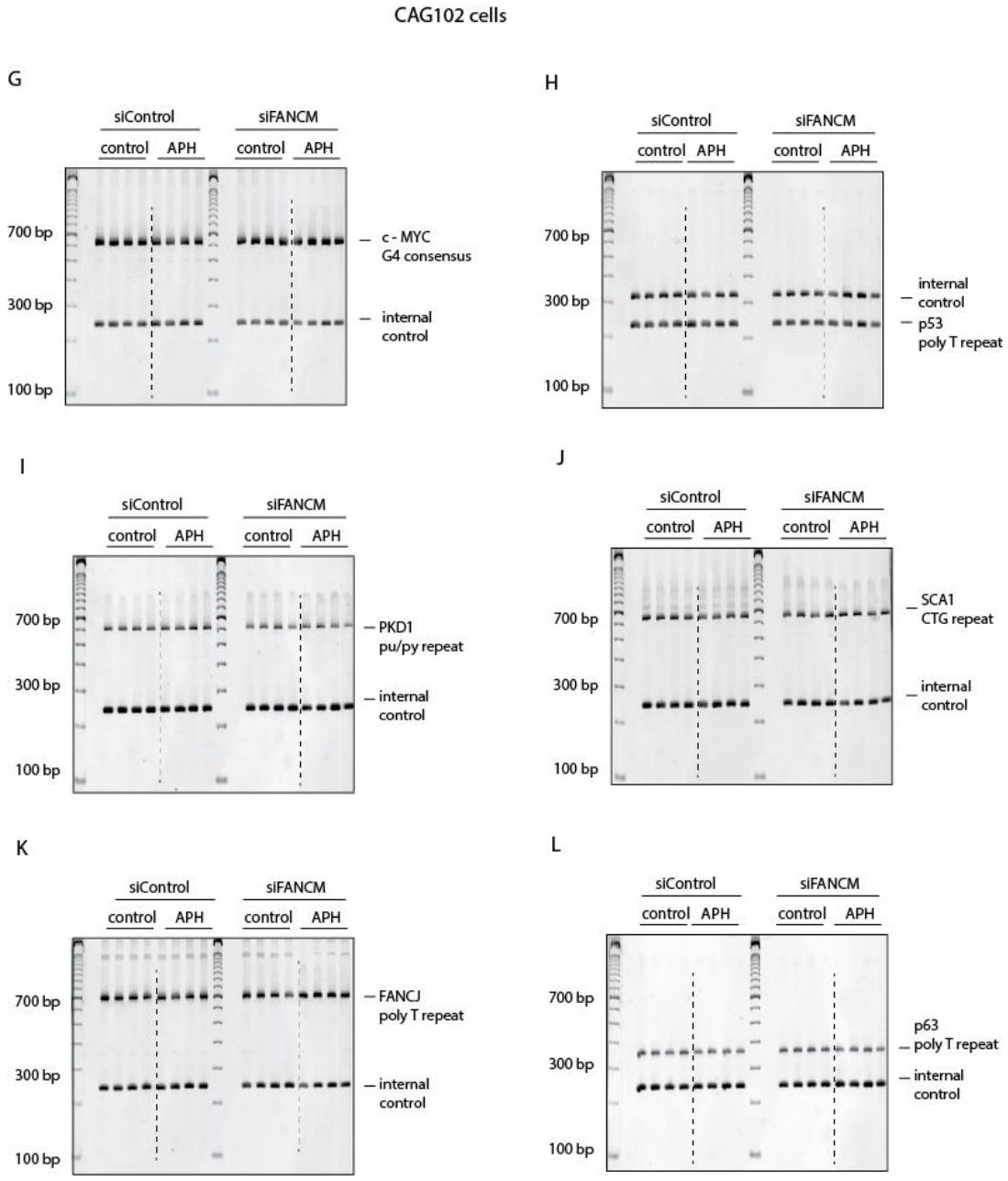

Figure 24 (continued). FANCM knockdown does not lead to loss of PCR signal at multiple endogenous sites with aphidicolin treatment. Small pool PCR results of CAG_{102} cells treated with siFANCM and aphidicolin. No loss of PCR signal was observed at multiple endogenous microsatellites.

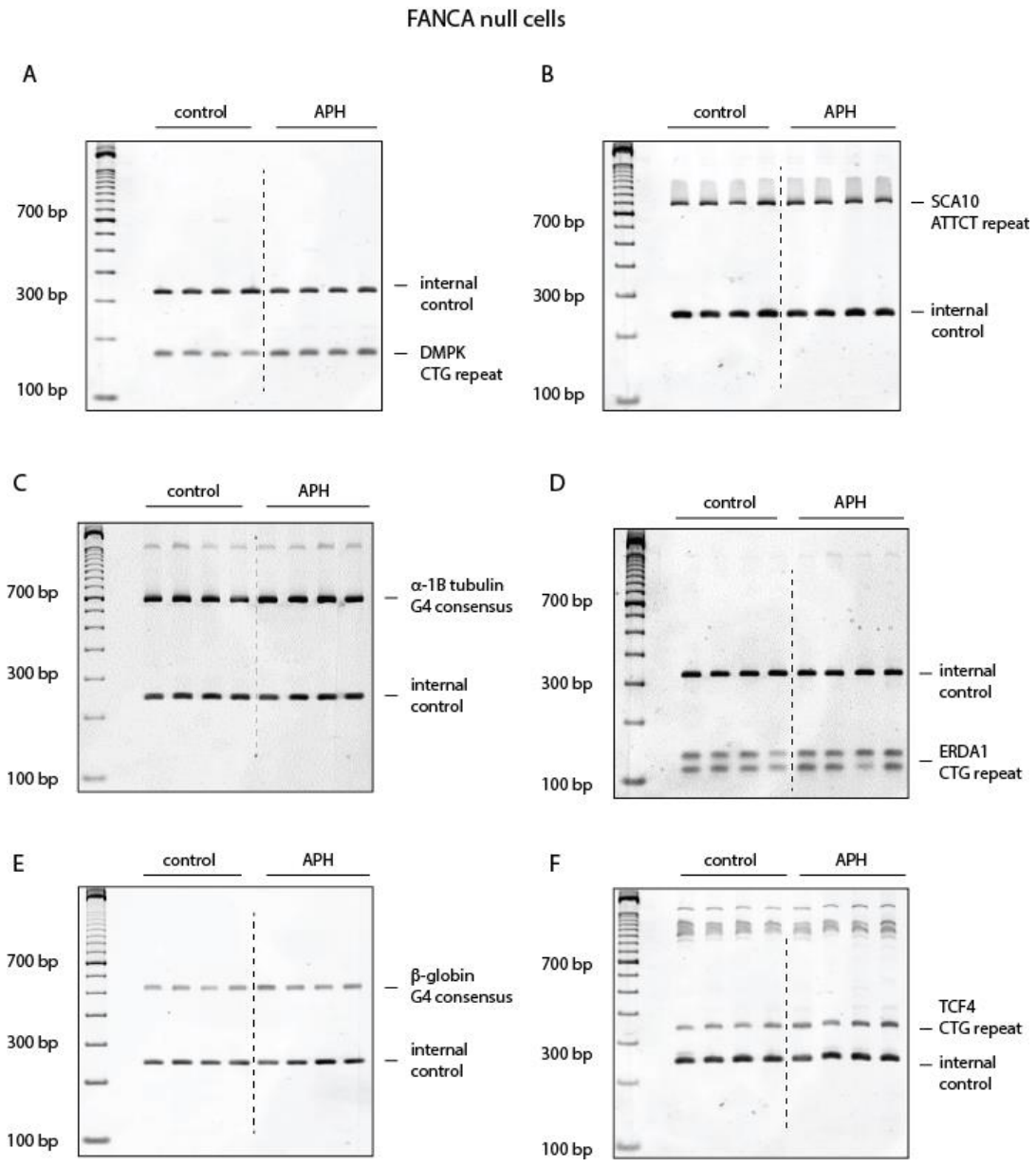

Figure 25. Aphidicolin treatment of FANCA nulls cells. Multiple endogenous microsatellites were examined via small pool PCR after aphidicolin treatment in FANCA null cells.

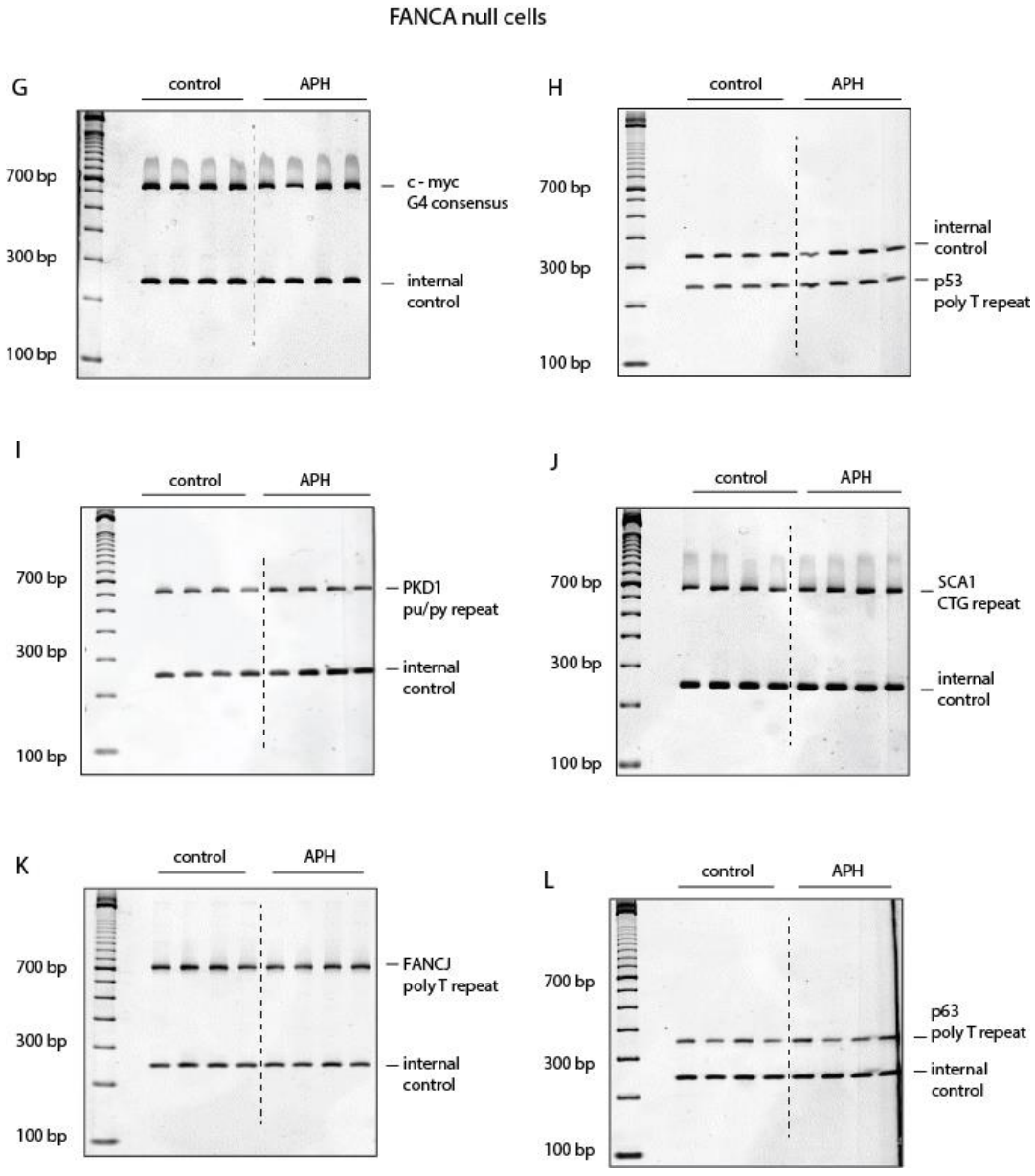

Figure 25 (continued). Aphidicolin treatment of FANCA nulls cells. Multiple endogenous microsatellites were examined via small pool PCR after aphidicolin treatment in FANCA null cells.

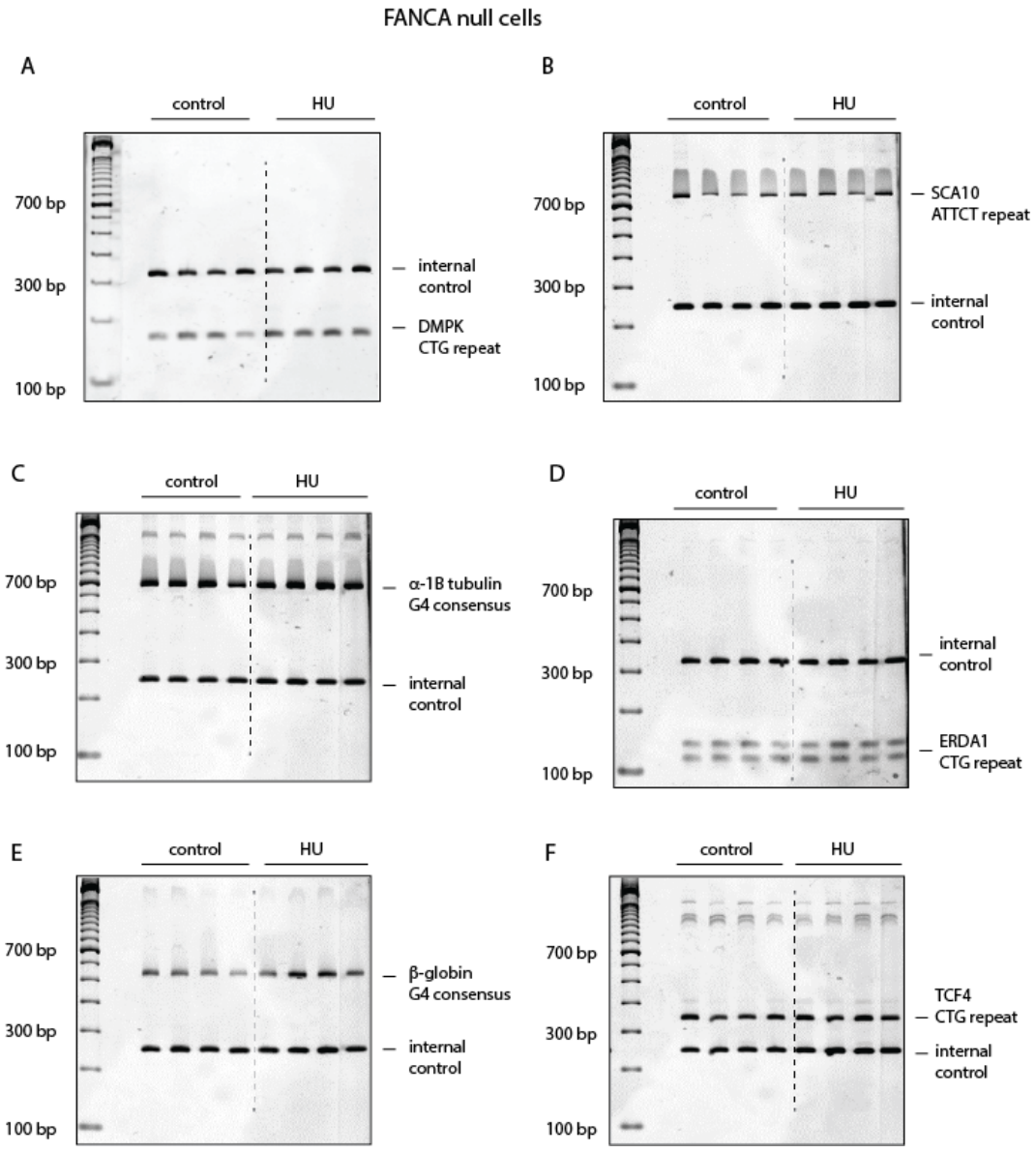

Figure 26. Hydroxyurea treatment of FANCA nulls cells. Multiple endogenous microsatellites were examined via small pool PCR after hydroxyurea treatment in FANCA null cells.

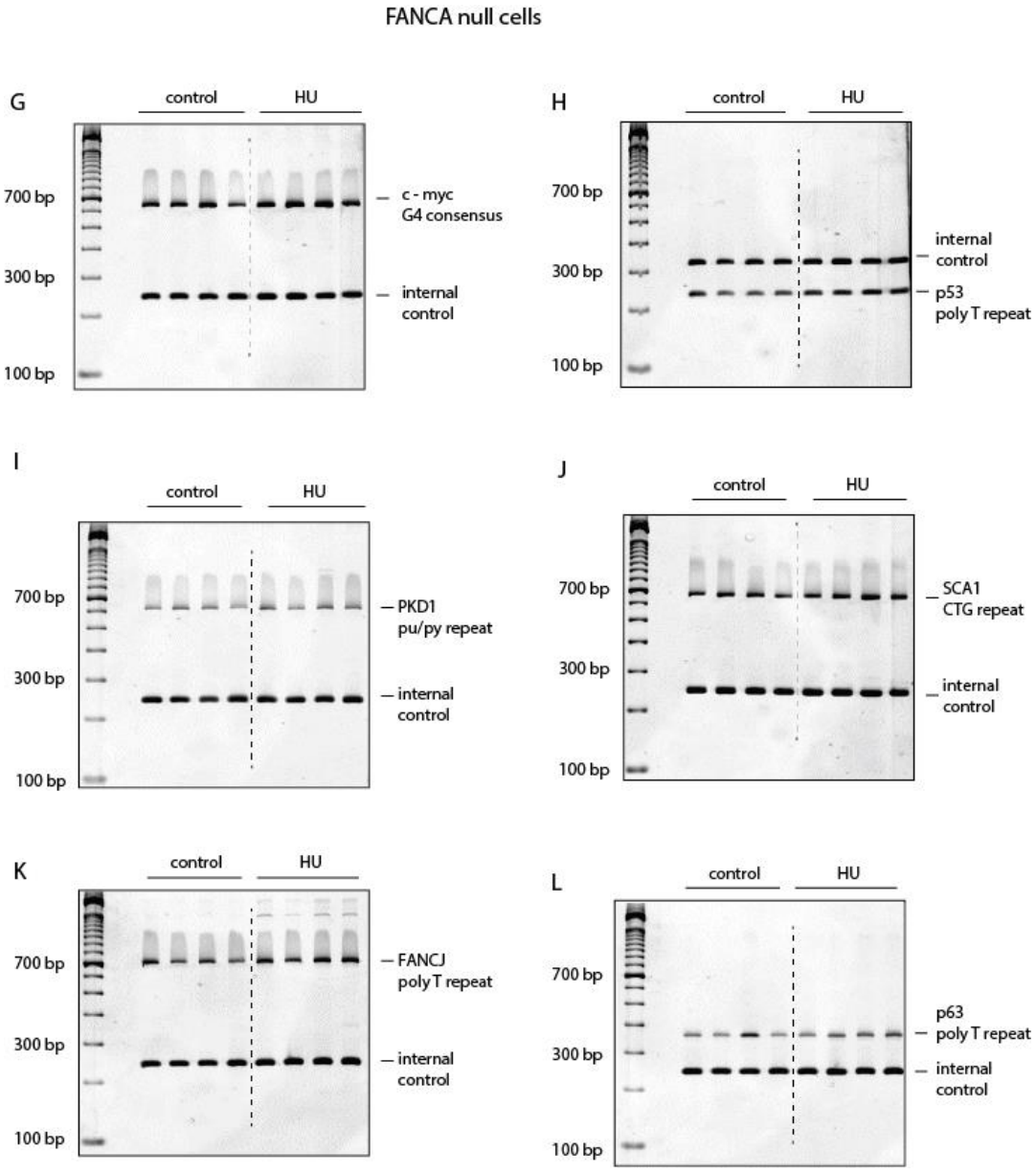

Figure 26 (continued). Hydroxyurea treatment of FANCA nulls cells. Multiple endogenous microsatellites were examined via small pool PCR after hydroxyurea treatment in FANCA null cells.

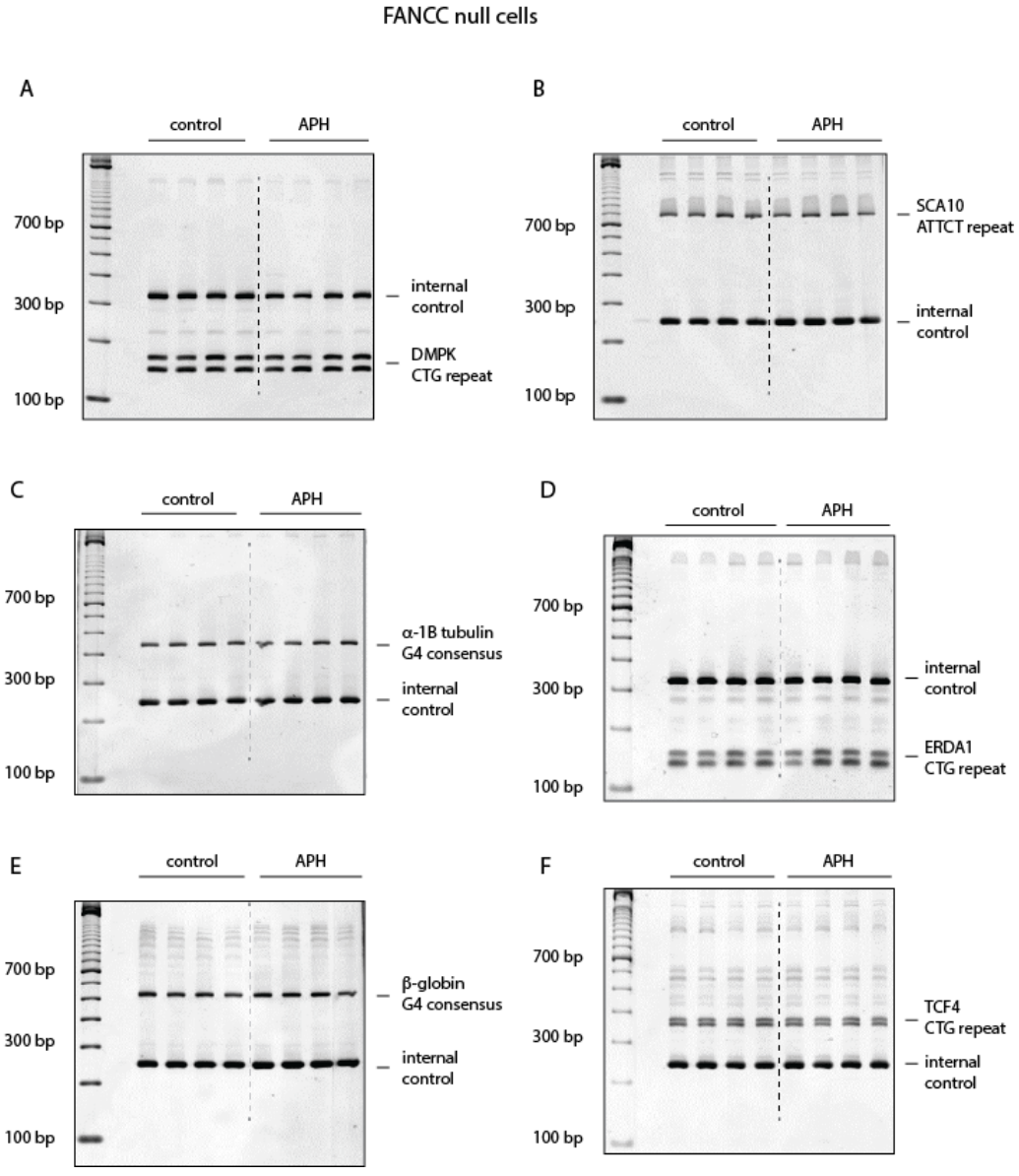

Figure 27. Aphidicolin treatment of FANCC nulls cells. Small pool PCR across repeated sequence loci in FANCC null cells treated with aphidicolin.

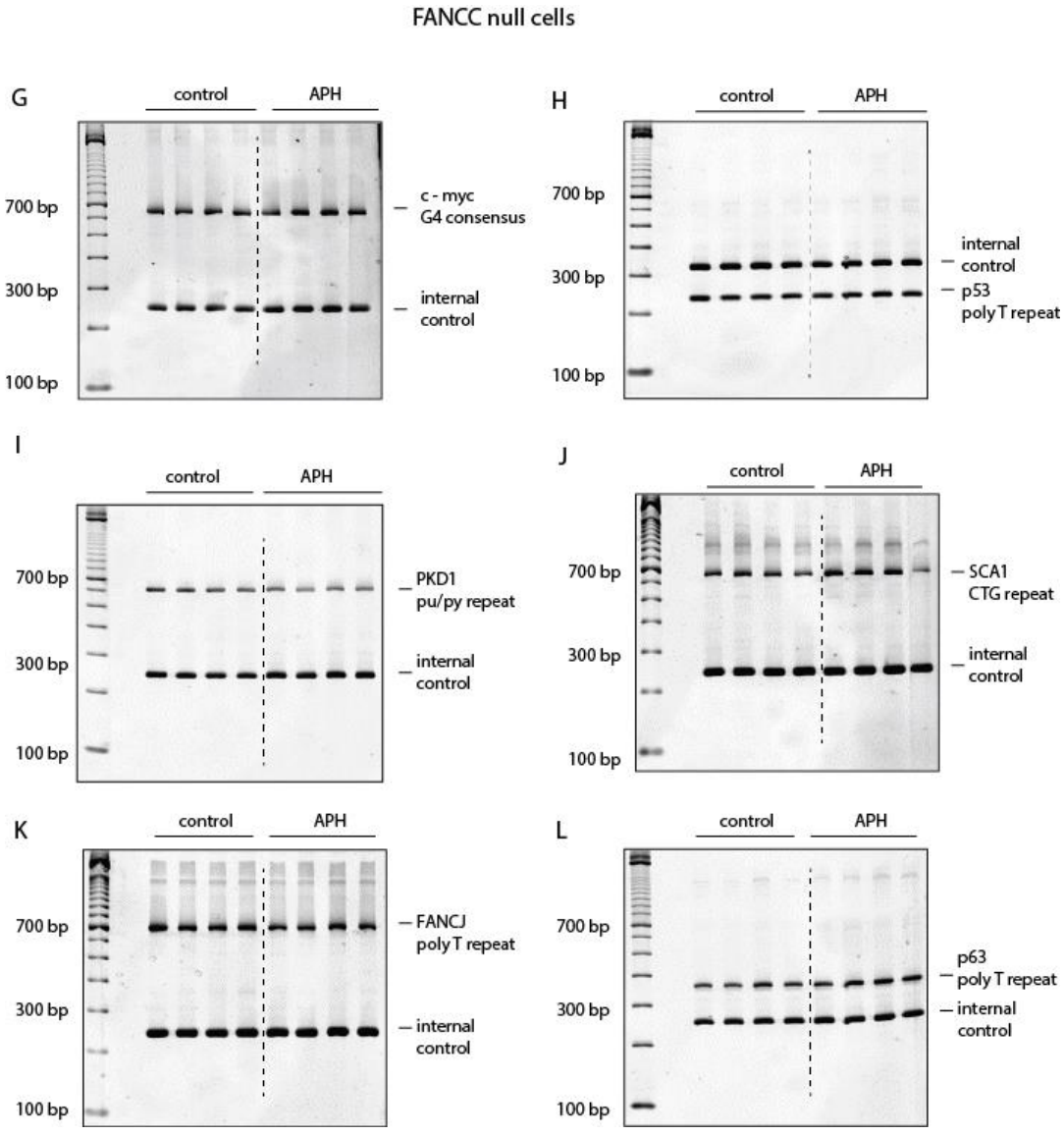

Figure 27 (continued). Aphidicolin treatment of FANCC nulls cells. Small pool PCR across repeated sequence loci in FANCC null cells treated with aphidicolin.

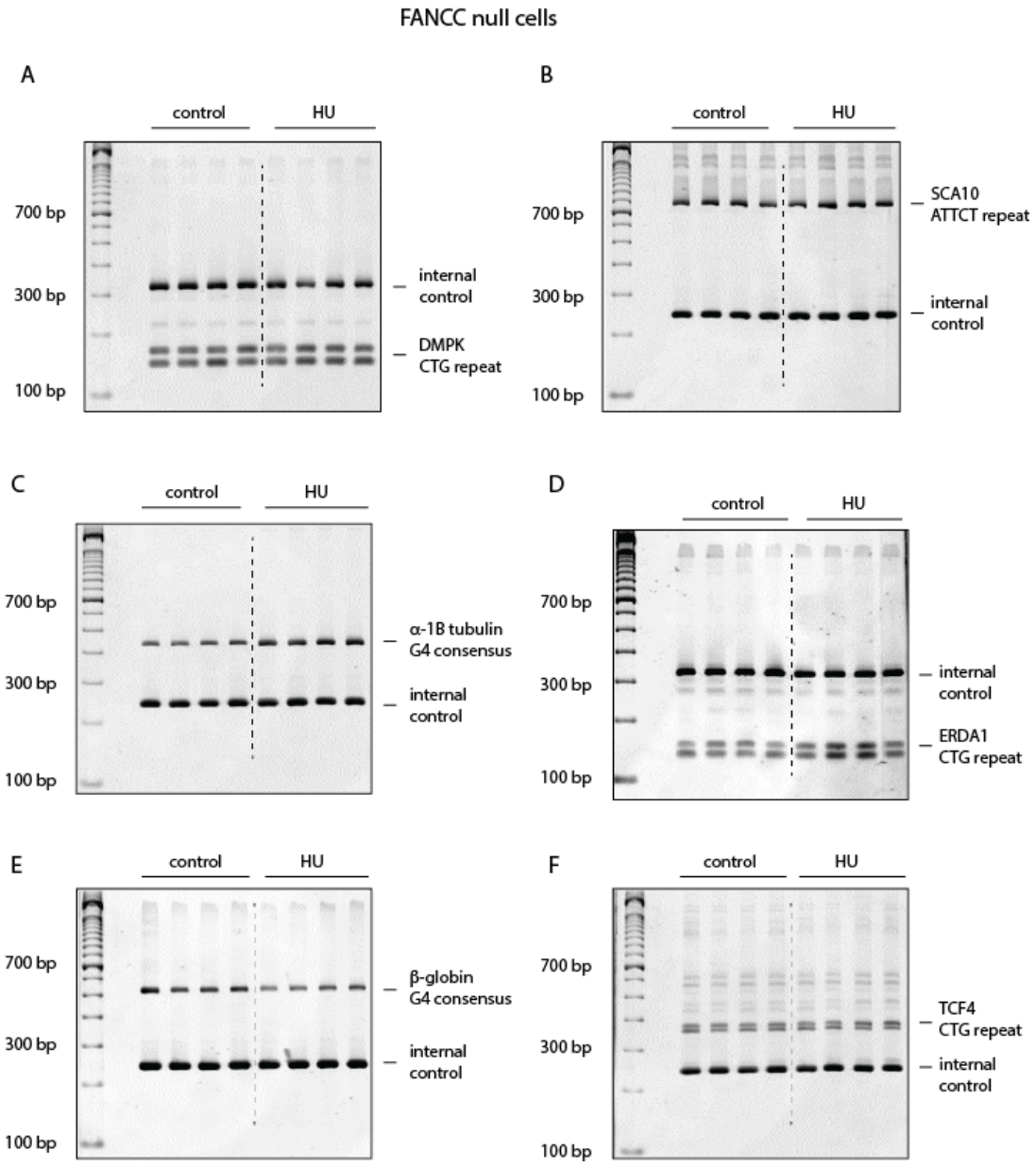

Figure 28. Hydroxyurea treatment of FANCC nulls cells. Small pool PCR across repeated sequence loci in FANCC null cells treated with hydroxyurea.

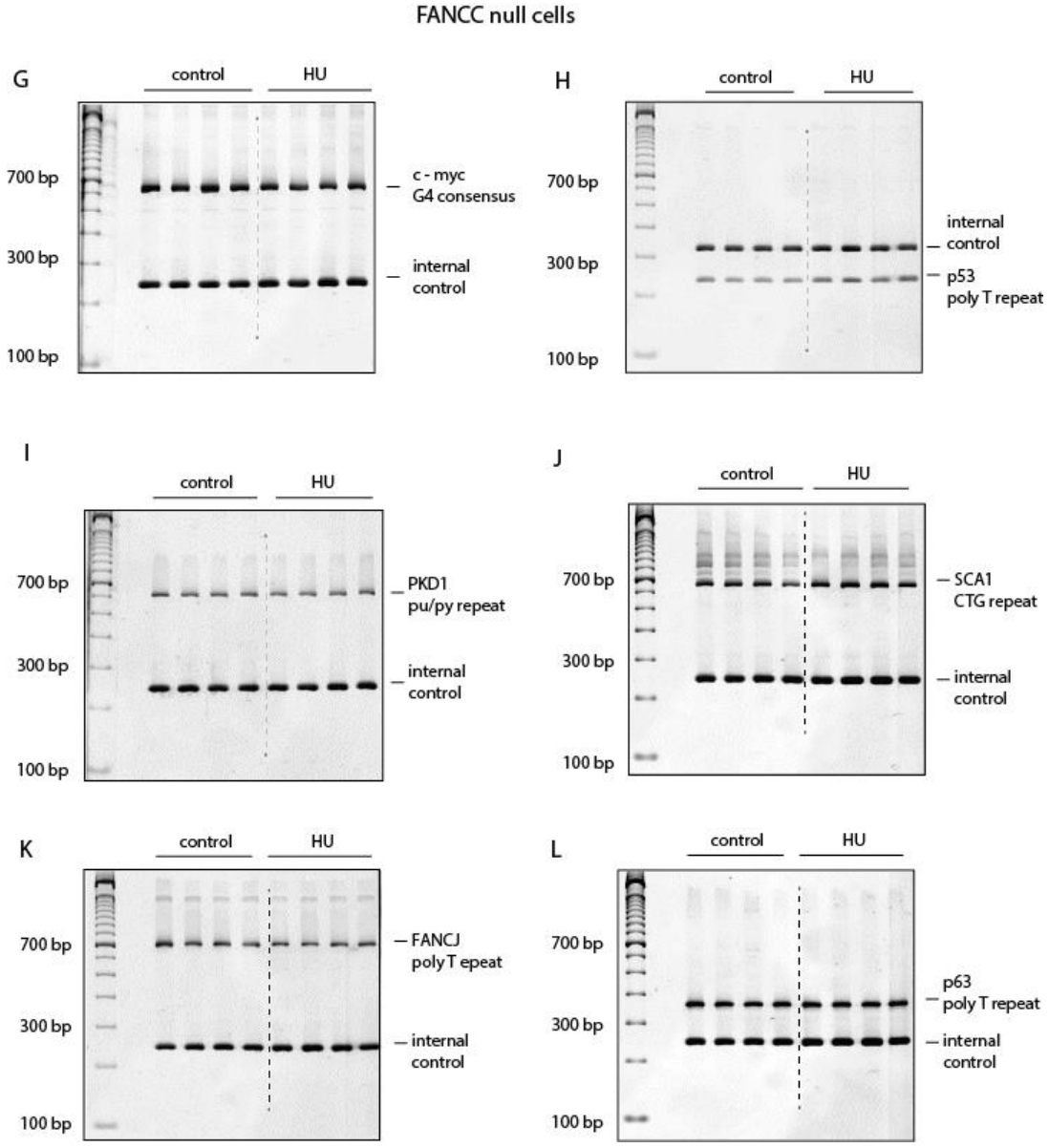

Figure 28 (continued). Hydroxyurea treatment of FANCC nulls cells. Small pool PCR across repeated sequence loci in FANCC null cells treated with hydroxyurea.

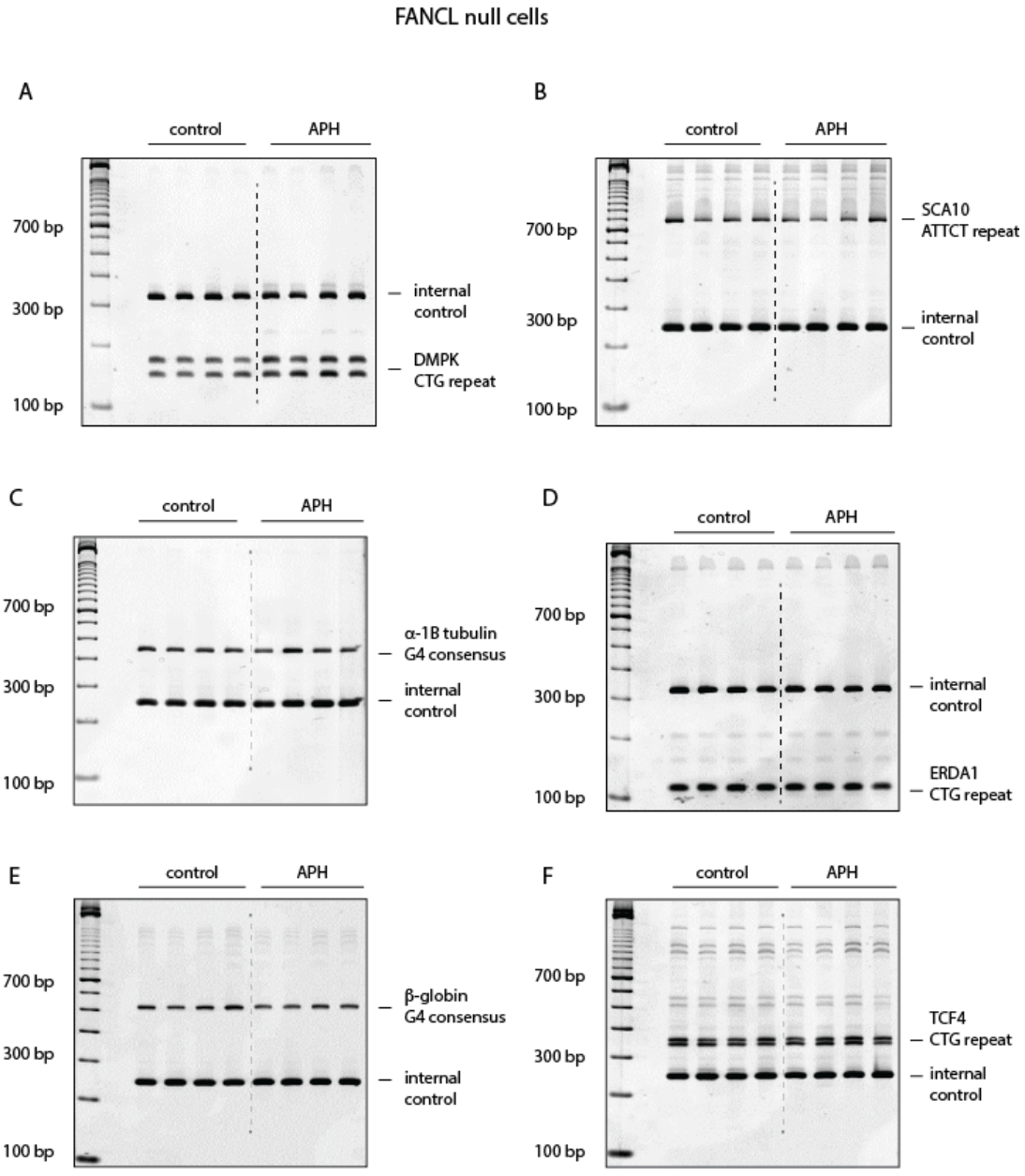

Figure 29. Aphidicolin treatment of FANCL nulls cells. Multiple endogenous microsatellites were examined via small pool PCR after aphidicolin treatment in FANCL null cells.

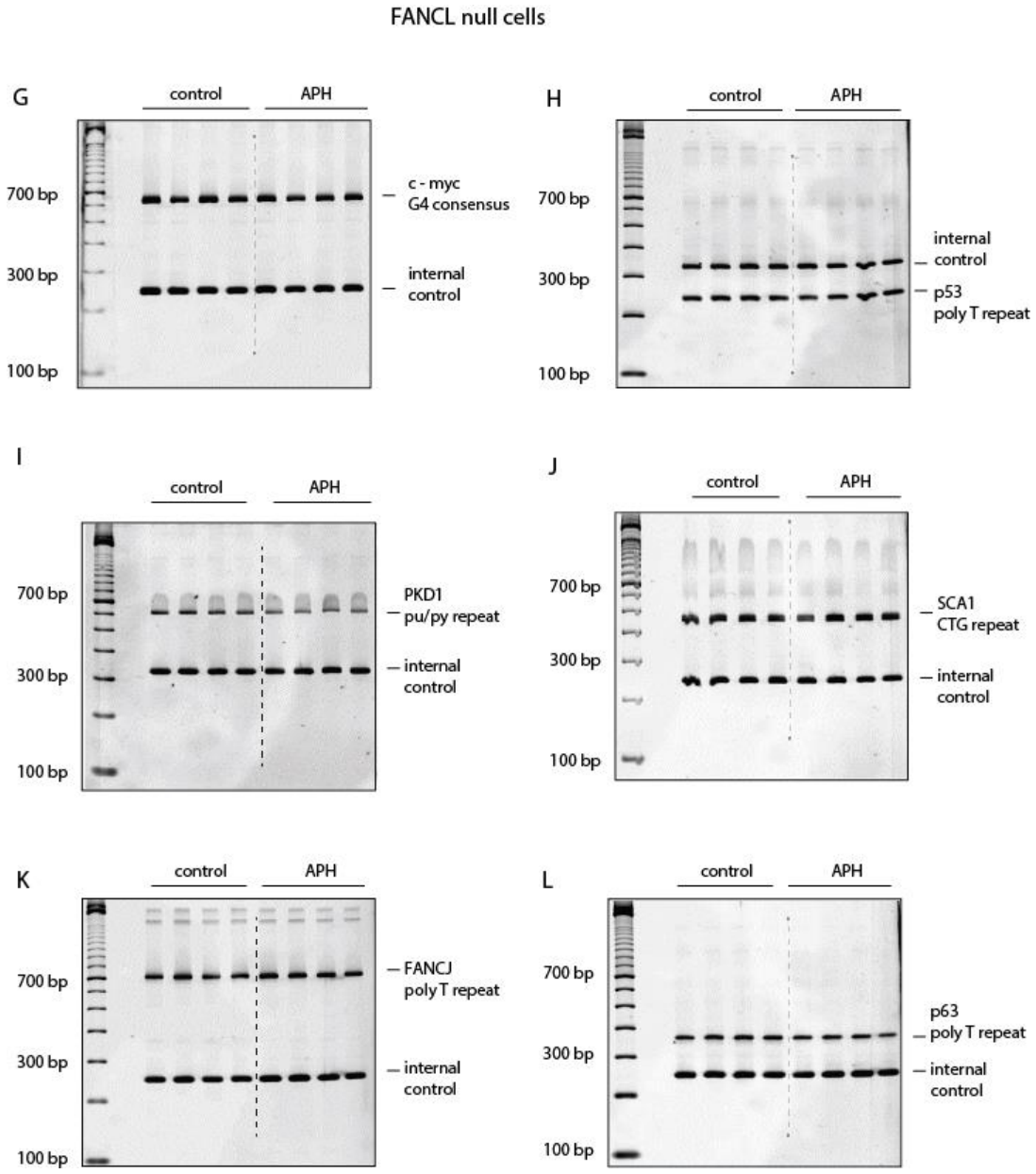

Figure 29 (continued). Aphidicolin treatment of FANCL nulls cells. Multiple endogenous microsatellites were examined via small pool PCR after aphidicolin treatment in FANCL null cells.

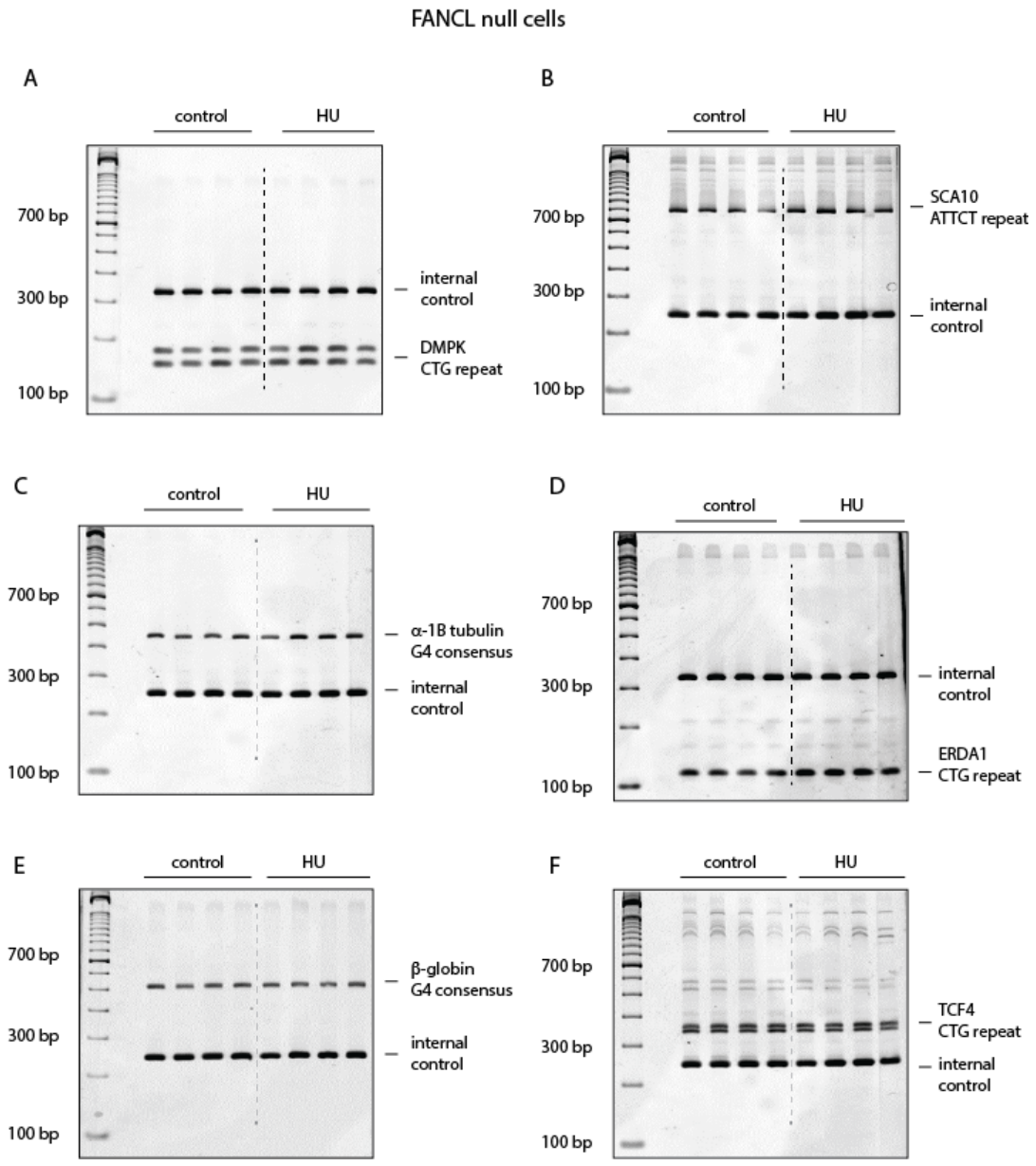

Figure 30. Hydroxyurea treatment of FANCL nulls cells. Multiple endogenous microsatellites were examined via small pool PCR after hydroxyurea treatment in FANCL null cells.

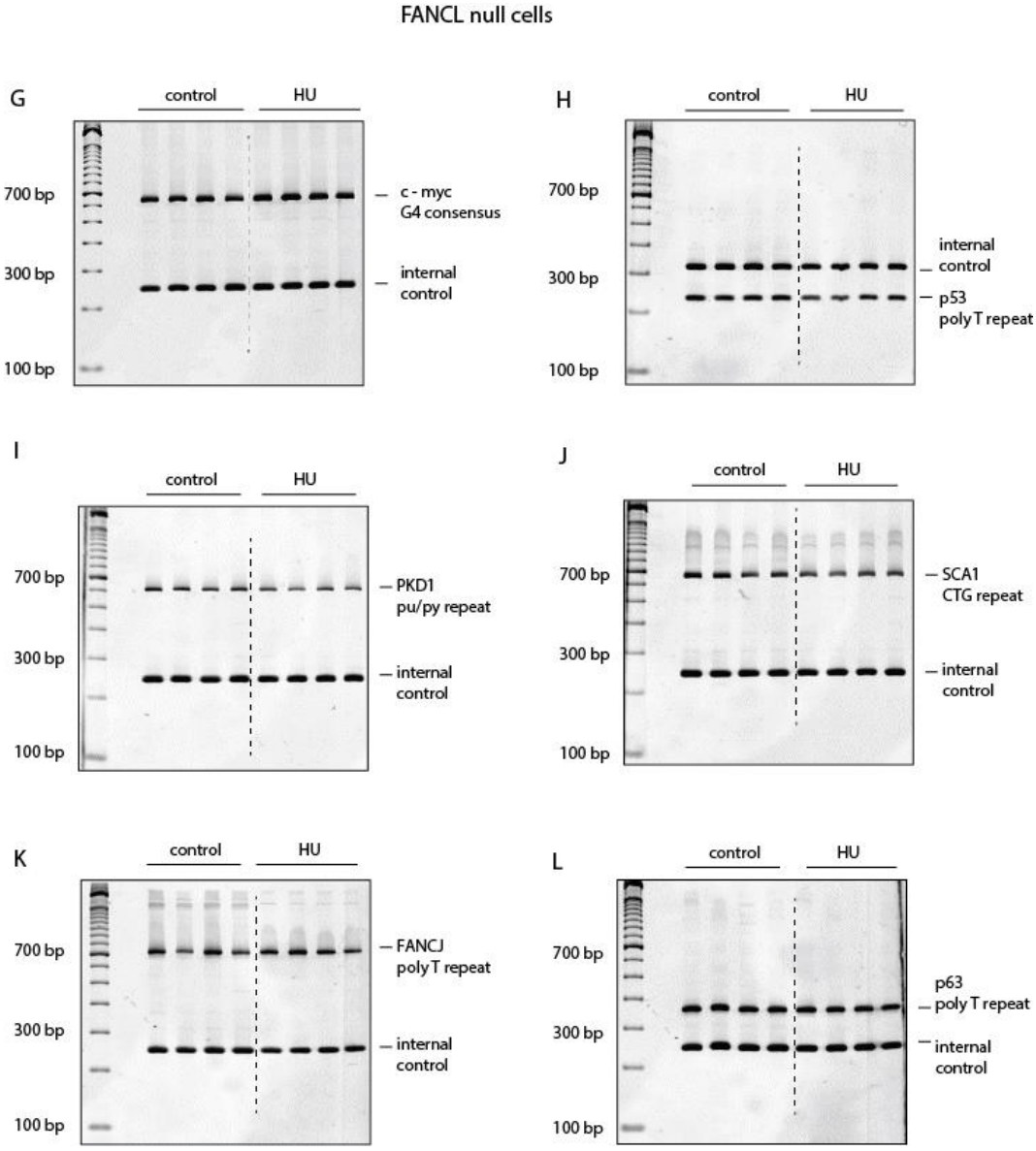

Figure 30 (continued). Hydroxyurea treatment of FANCL nulls cells. Multiple endogenous microsatellites were examined via small pool PCR after hydroxyurea treatment in FANCL null cells.

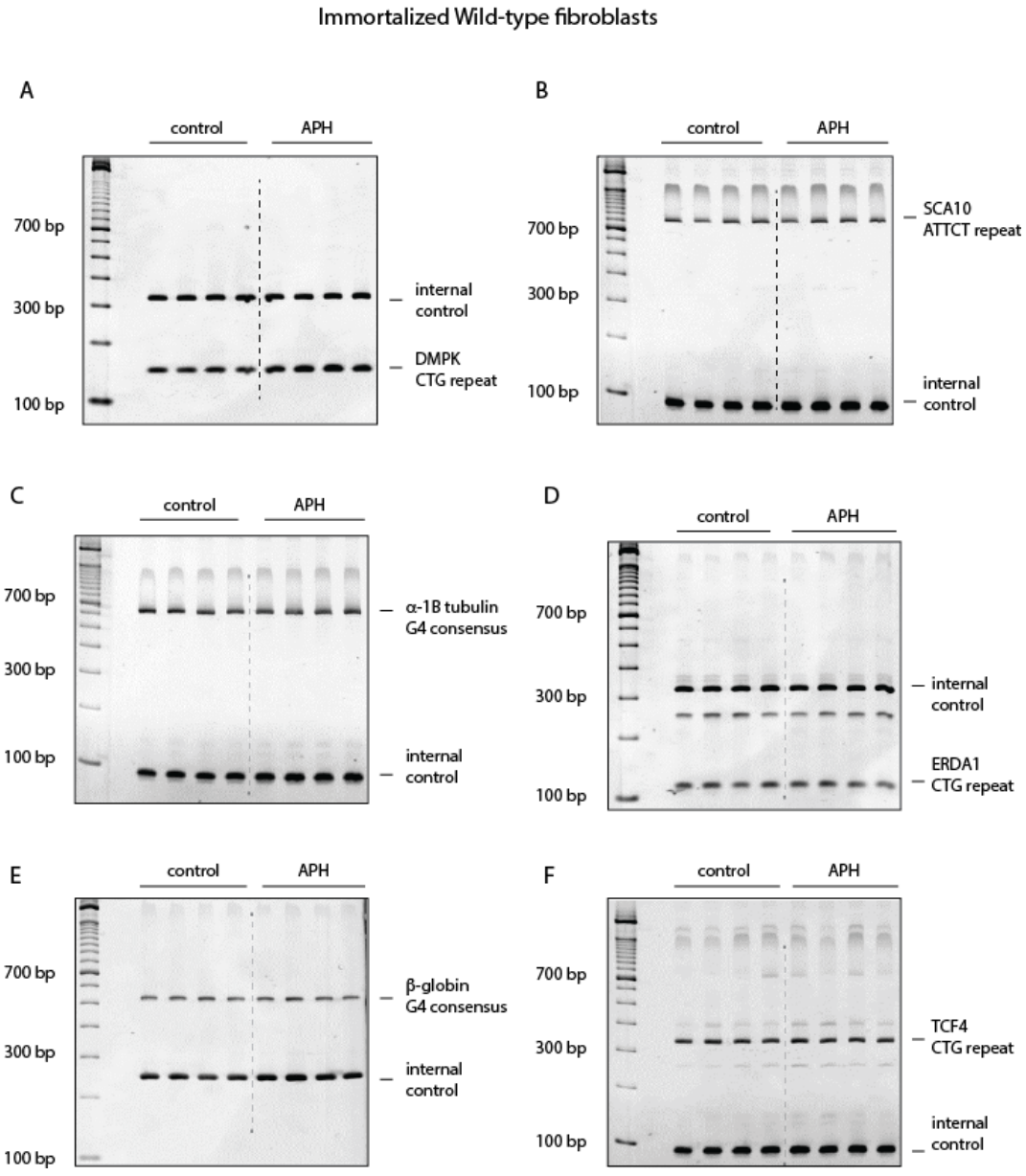

Figure 31. Aphidicolin treatment of immortalized wild-type fibroblast cells. Small pool PCR across repeated sequence in DNA from immortalized wild-type fibroblast cells treated with or without aphidicolin.

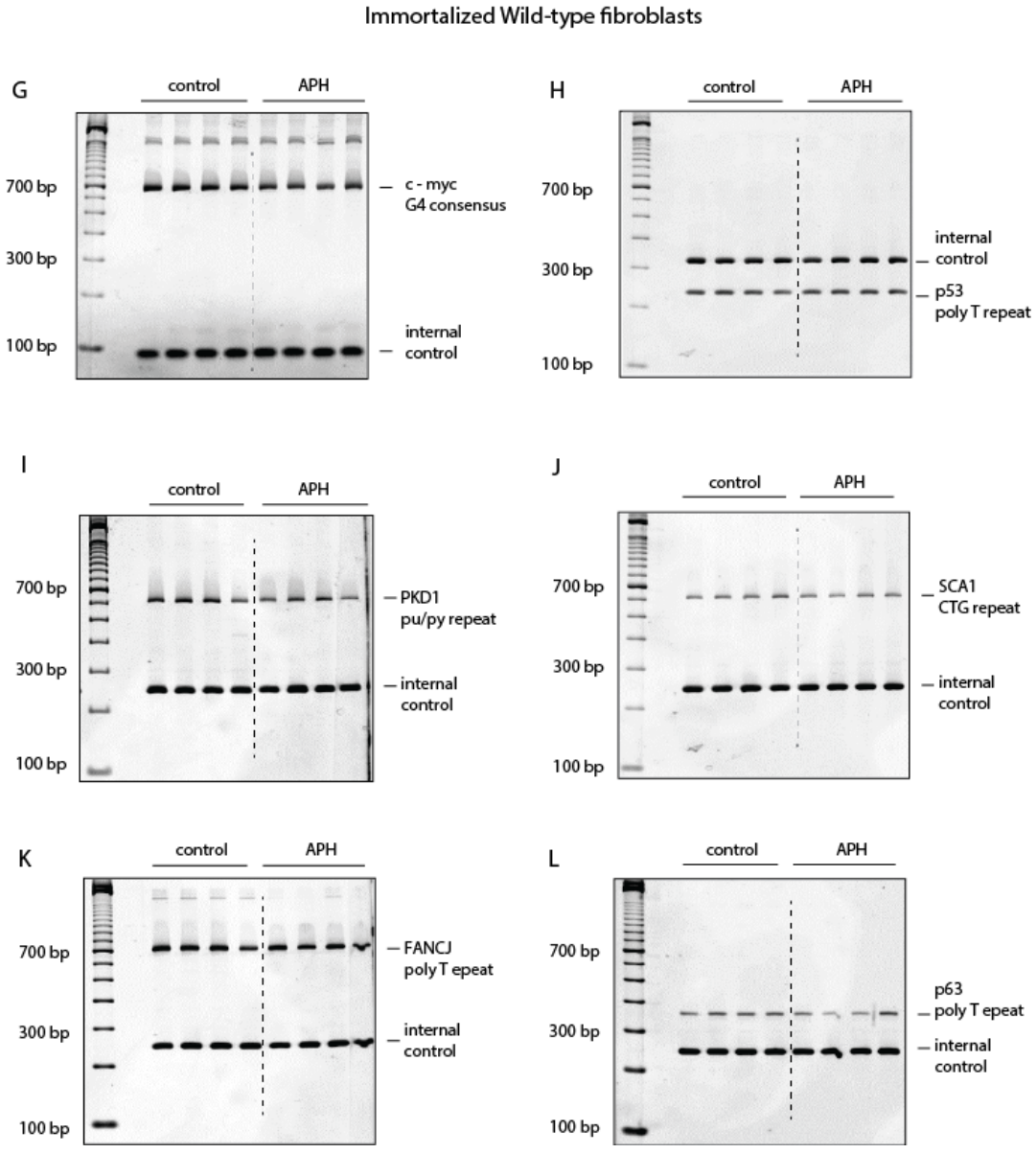

Figure 31 (continued). Aphidicolin treatment of immortalized wild-type fibroblast cells. Small pool PCR across repeated sequence in DNA from immortalized wild-type fibroblast cells treated with or without aphidicolin.

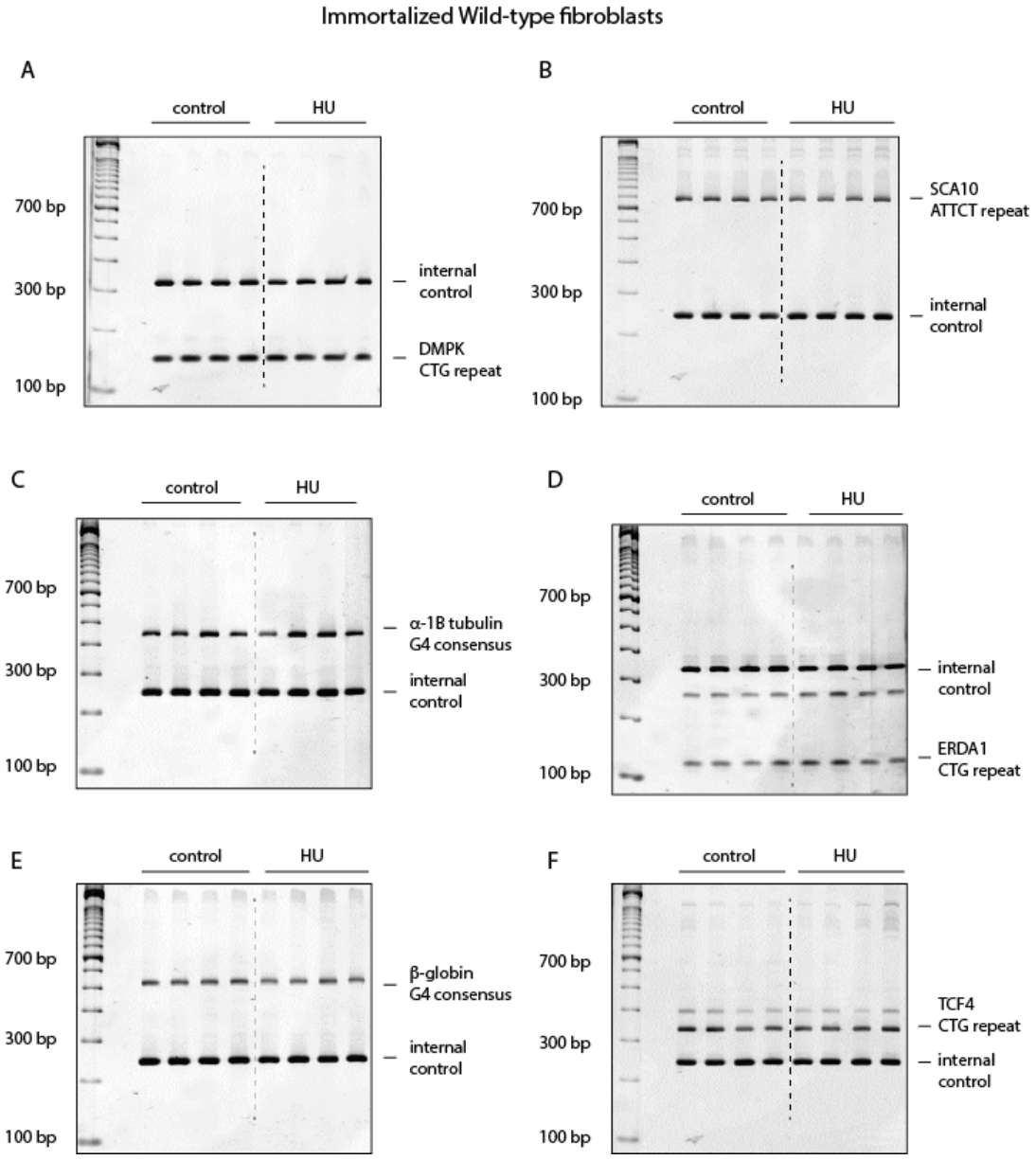

Figure 32. Hydroxyurea treatment of immortalized wild-type fibroblast cells. Small pool PCR across repeated sequence in DNA from immortalized wild-type fibroblast cells treated with or without hydroxyurea.

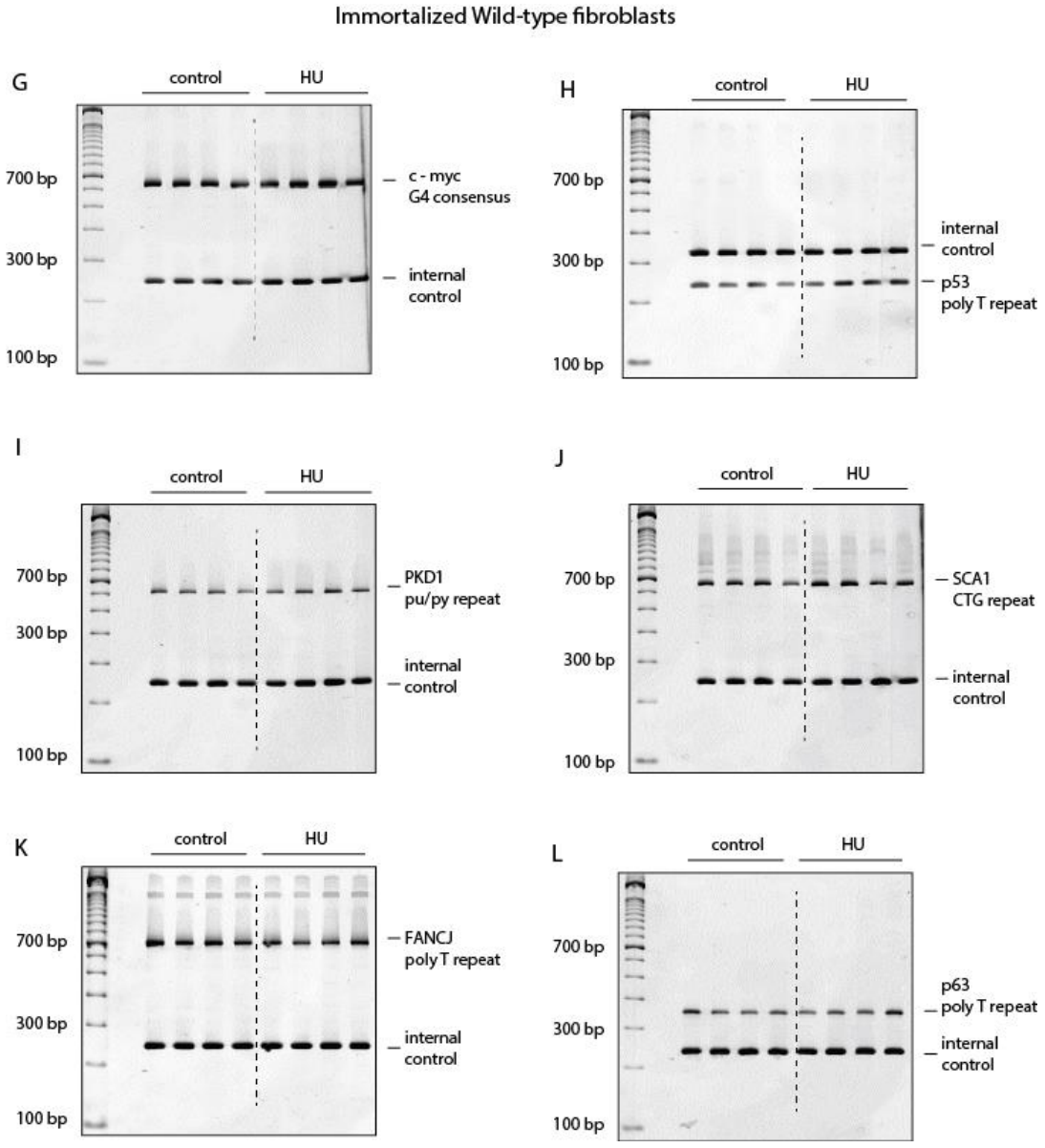

Figure 32 (continued). Hydroxyurea treatment of wild-type fibroblast cells. Small pool PCR across repeated sequence in DNA from wild-type fibroblast cells treated with or without hydroxyurea.

33). These data support the conclusion that FANCJ stabilizes microsatellite sequences regardless of FA core complex assembly.

The signal for FANCJ recruitment to the site of DNA damage during a FA repair pathway response is heterodimer formation of monoubiquitinated FANCD2 and FANCI. Knockdown of FANCD2 using a pool of four siRNAs in $CTG_{102}{\cdot}CAG_{102}$ cells treated with aphidicolin did not lead to a loss of PCR signal at either the (CTG)·(CAG) ectopic site repeats (Figure 33) or endogenous microsatellites (Figure 34) suggesting FANCJ works independently of the FA repair pathway to stabilize microsatellite sequences. These results were confirmed in immortalized FANCD2 null patient cells treated with APH (Figure 35) or HU (Figure 36). In addition, immortalized FANCI null cells were treated with either APH (Figure 37) or HU (Figure 38). Examination of endogenous microsatellite sequences with spPCR showed no loss of PCR signal indicating FANCJ maintains microsatellite stability independent of FANCD2 and FANCI hetereodimer formation.

FANCJ is a downstream effector of the FA repair pathway. Other downstream effectors of the FA repair pathway participate in other DNA repair pathways including homologous recombination. We sought to determine if microsatellite stabilization is a function of these proteins as well. FANCP/SLX4 (Figure 39 and 40), FANCQ/ERCC4 (Figure 41 and 42), and FANCD1/BRCA2 (Figure 43 and 44) immortalized patient fibroblasts were subjected to replicative stress (APH or HU). Microsatellite instability was not detected in these cells after replication stress implying FANCJ has a unique role in microsatellite stabilization.

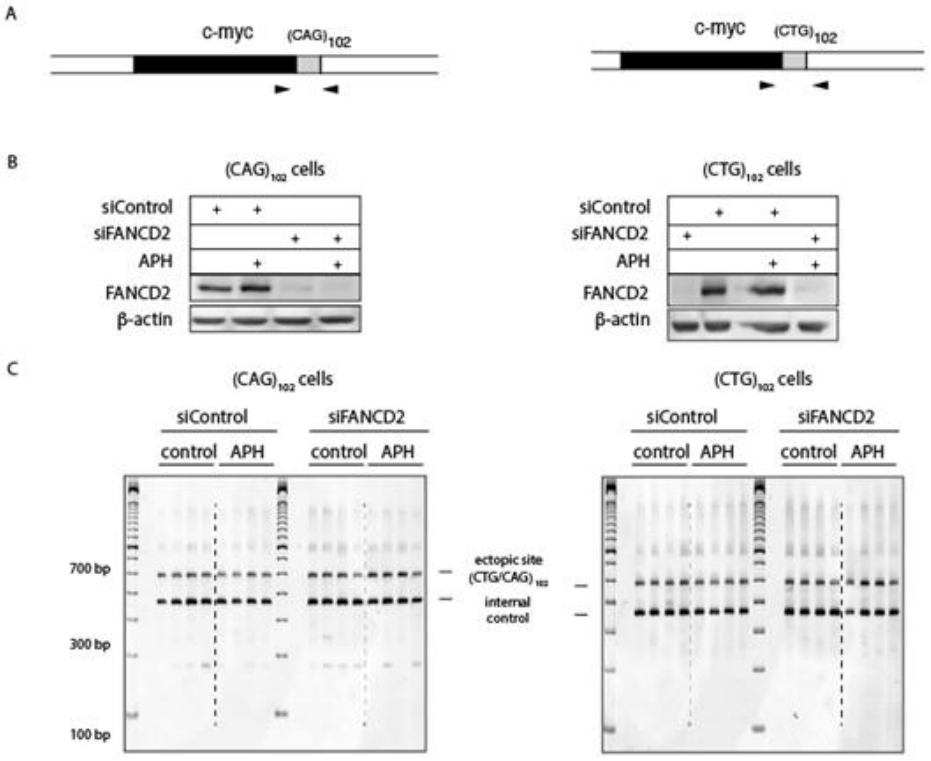

Figure 33. FANCD2 knockdown does not lead to loss of ectopic (CTG)·(CAG) microsatellite signal in $(CTG)_{102}$·$(CAG)_{102}$ cells treated with aphidicolin. **(A)** Diagram of the integration site in $(CAG)_{102}$·$(CTG)_{102}$ cell lines. **(B)** Whole cell extracts were isolated after treatment of cells with siRNA against FANCD2 and aphidicolin for a total of five transfections, or parallel untreated cultures, and immunoblotted for FANC D2. **(C)** Small pool PCR results with primers flanking the ectopic $(CAG)_{102}$·$(CTG)_{102}$ repeats and primers for a site without microsatellites serving as an internal control.

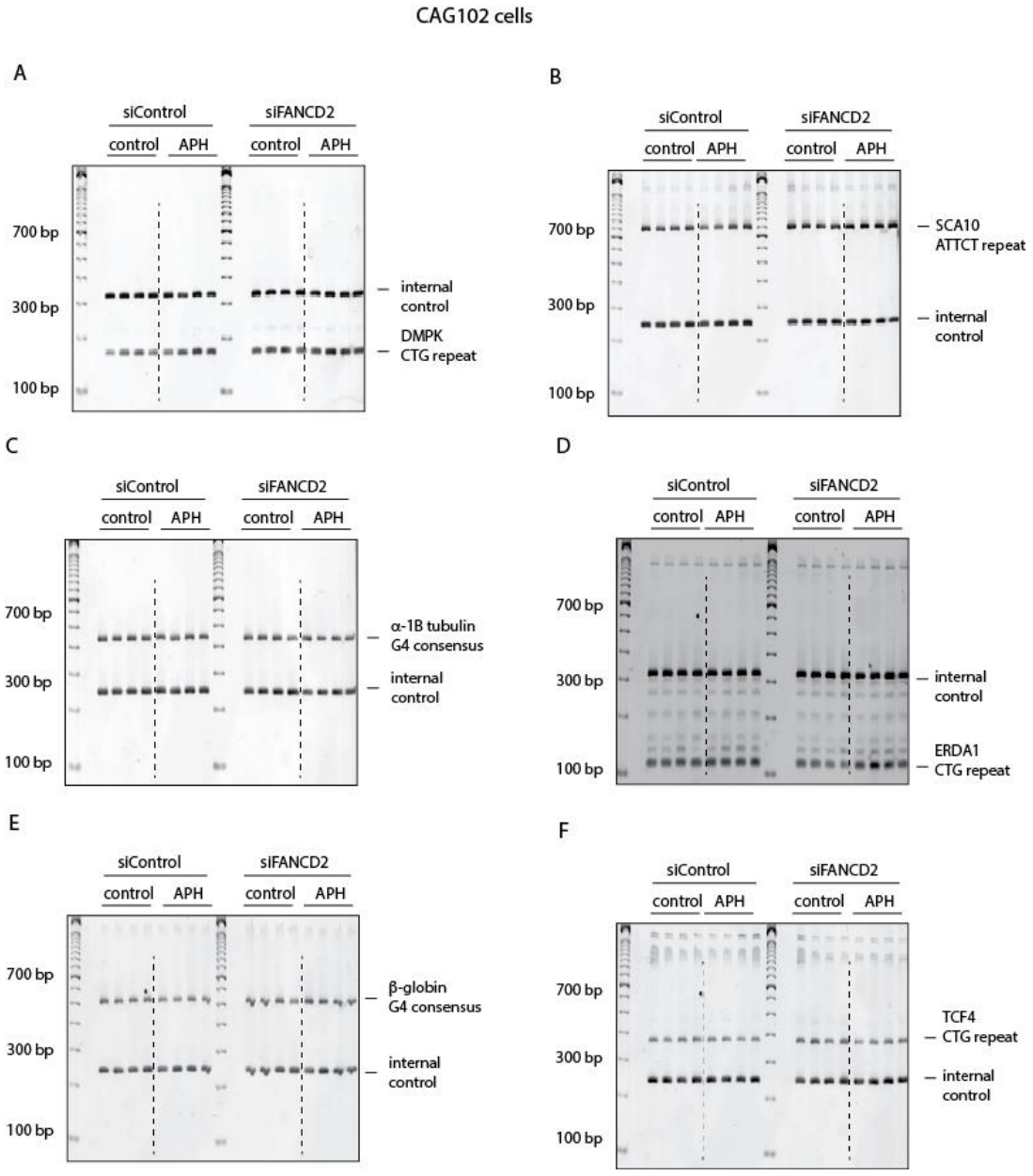

Figure 34. FANCD2 knockdown does not lead to loss of PCR signal at multiple endogenous sites with aphidicolin treatment. Small pool PCR results of CAG_{102} cells treated with siFANCD2 and aphidicolin showing no patterns of instability at multiple endogenous microsatellites.

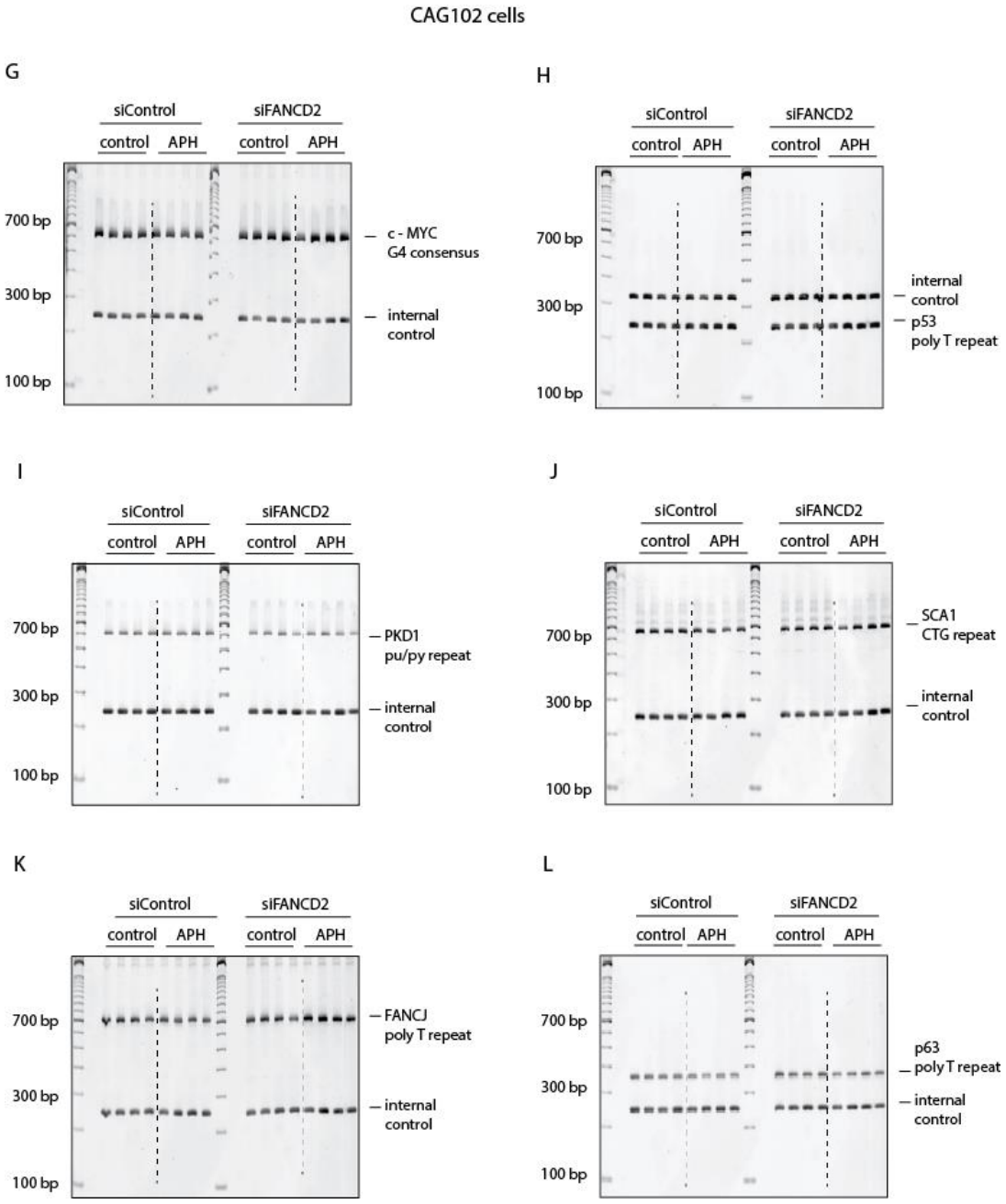

Figure 34 (continued). FANCD2 knockdown does not lead to loss of PCR signal at multiple endogenous sites with aphidicolin treatment. Small pool PCR results of CAG_{102} cells treated with siFANCD2 and aphidicolin showing no patterns of instability at multiple endogenous microsatellites.

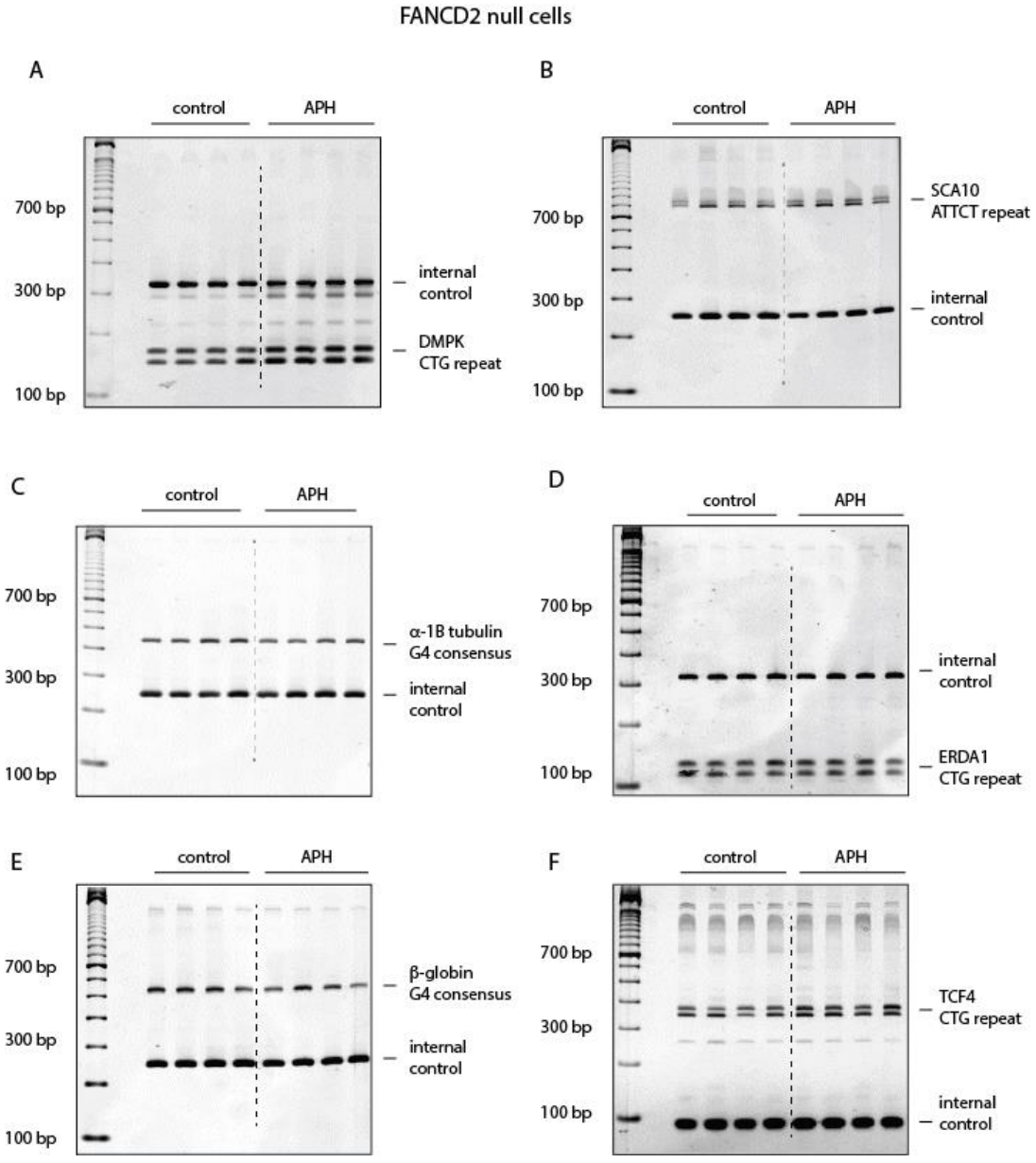

Figure 35. Aphidicolin treatment of FANCD2 null cells. Small pool PCR results of DNA from FANCD2 null patient fibroblast cells treated with aphidicolin.

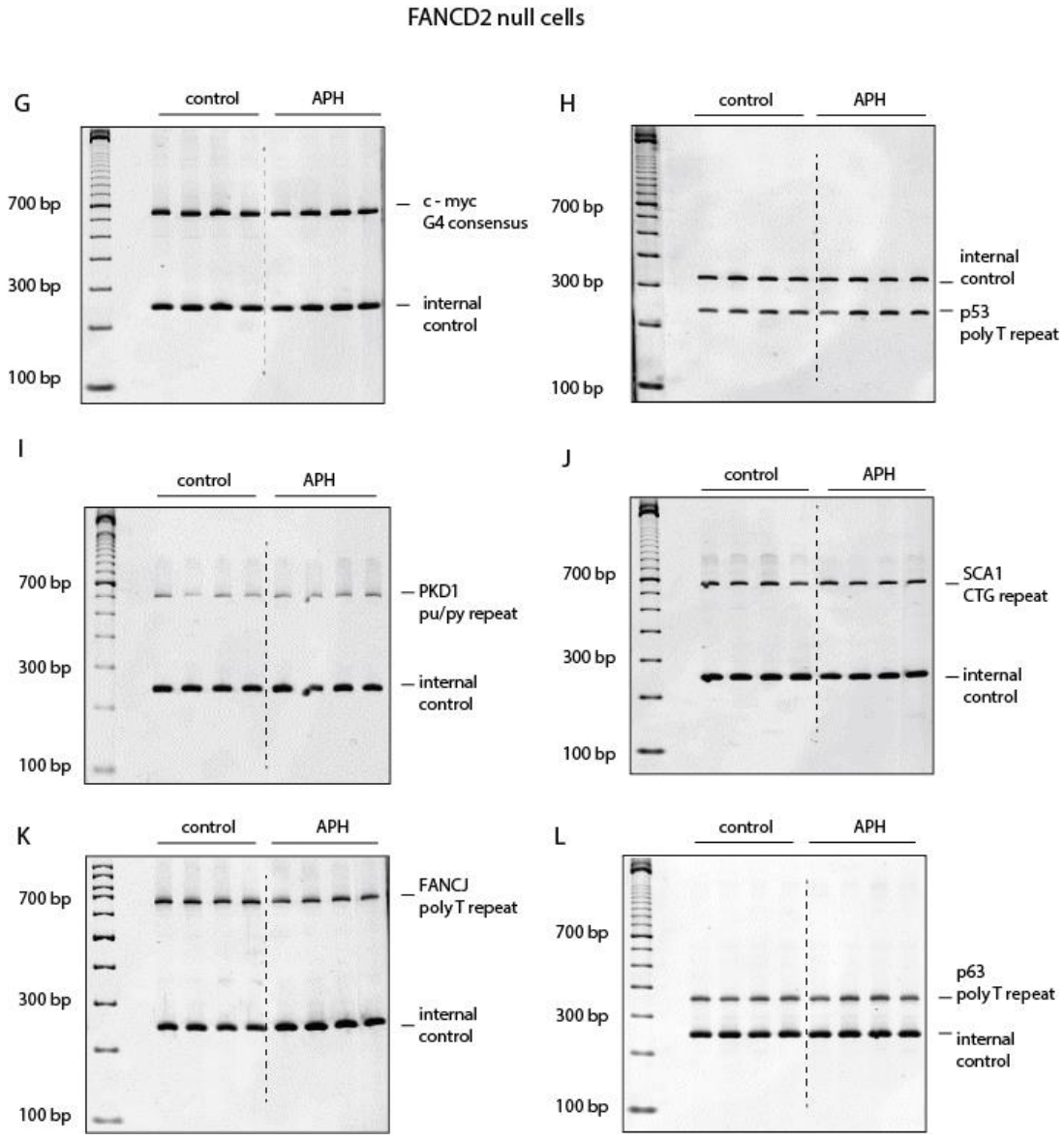

Figure 35 (continued). Aphidicolin treatment of FANCD2 null cells. Small pool PCR results of DNA from FANCD2 null patient fibroblast cells treated with aphidicolin.

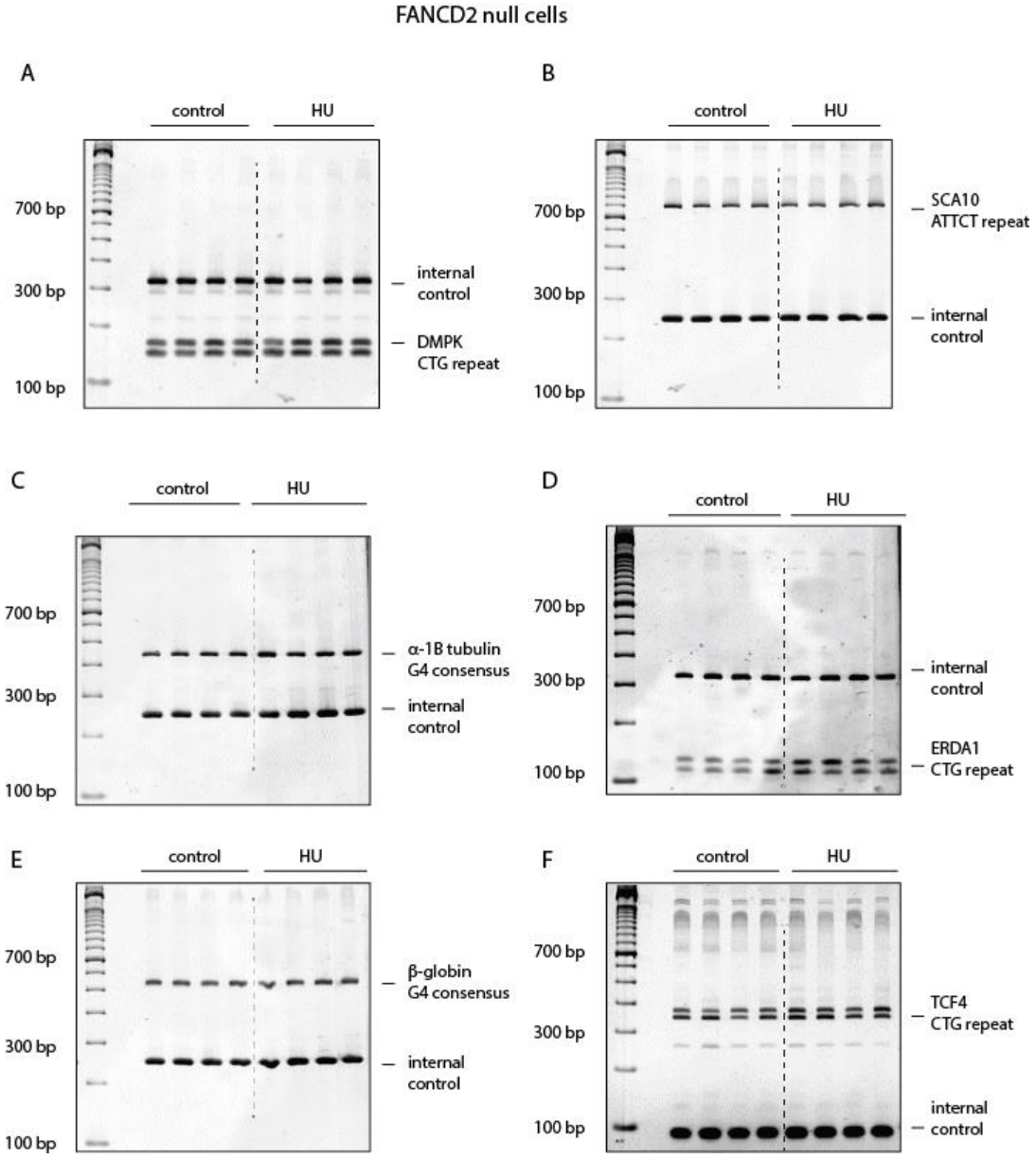

Figure 36. Hydroxyurea treatment of FANCD2 null cells. Small pool PCR results of DNA from FANCD2 null patient fibroblast cells treated with hydroxyurea.

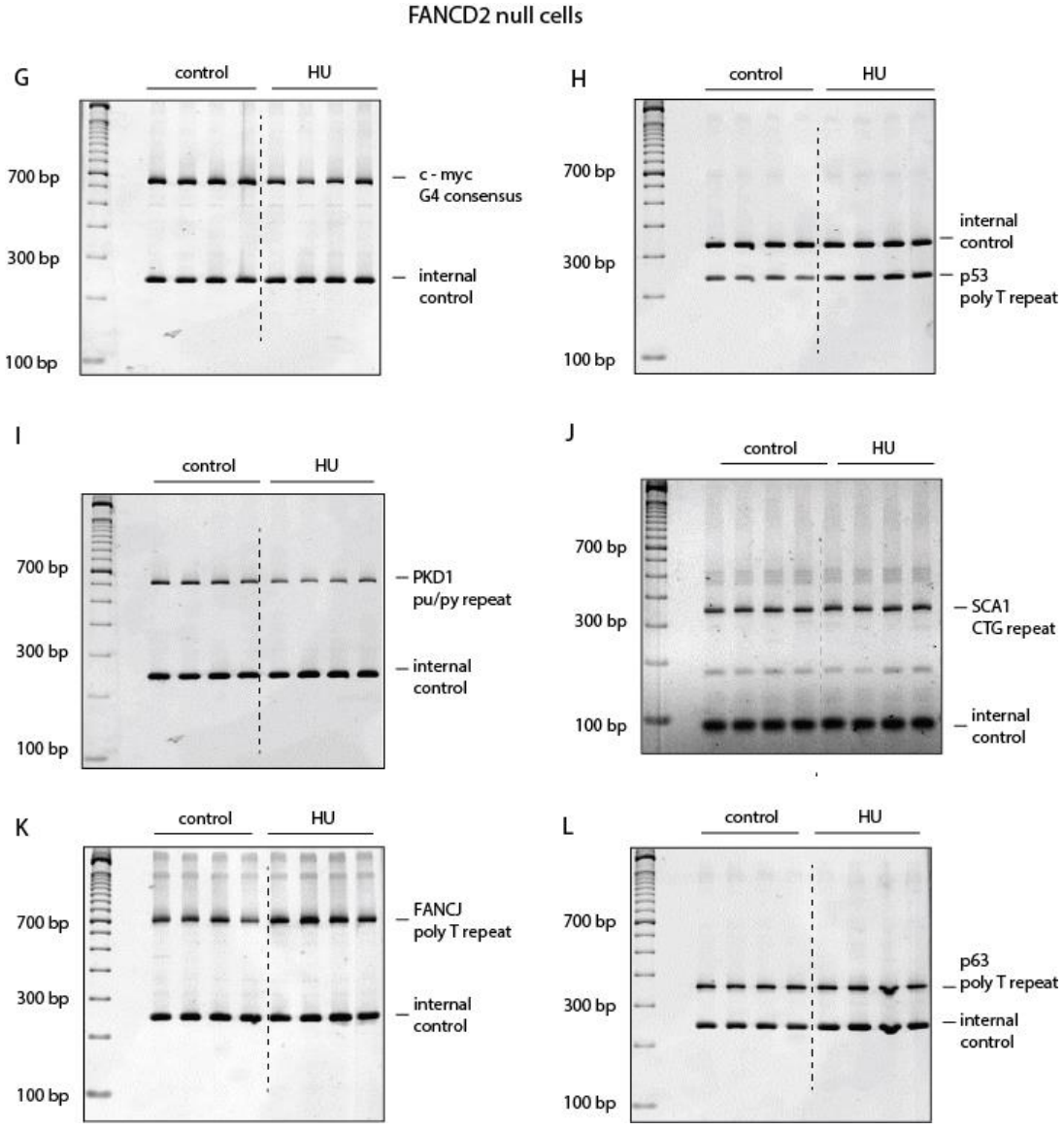

Figure 36 (continued). Hydroxyurea treatment of FANCD2 null cells. Small pool PCR results of DNA from FANCD2 null patient fibroblast cells treated with hydroxyurea.

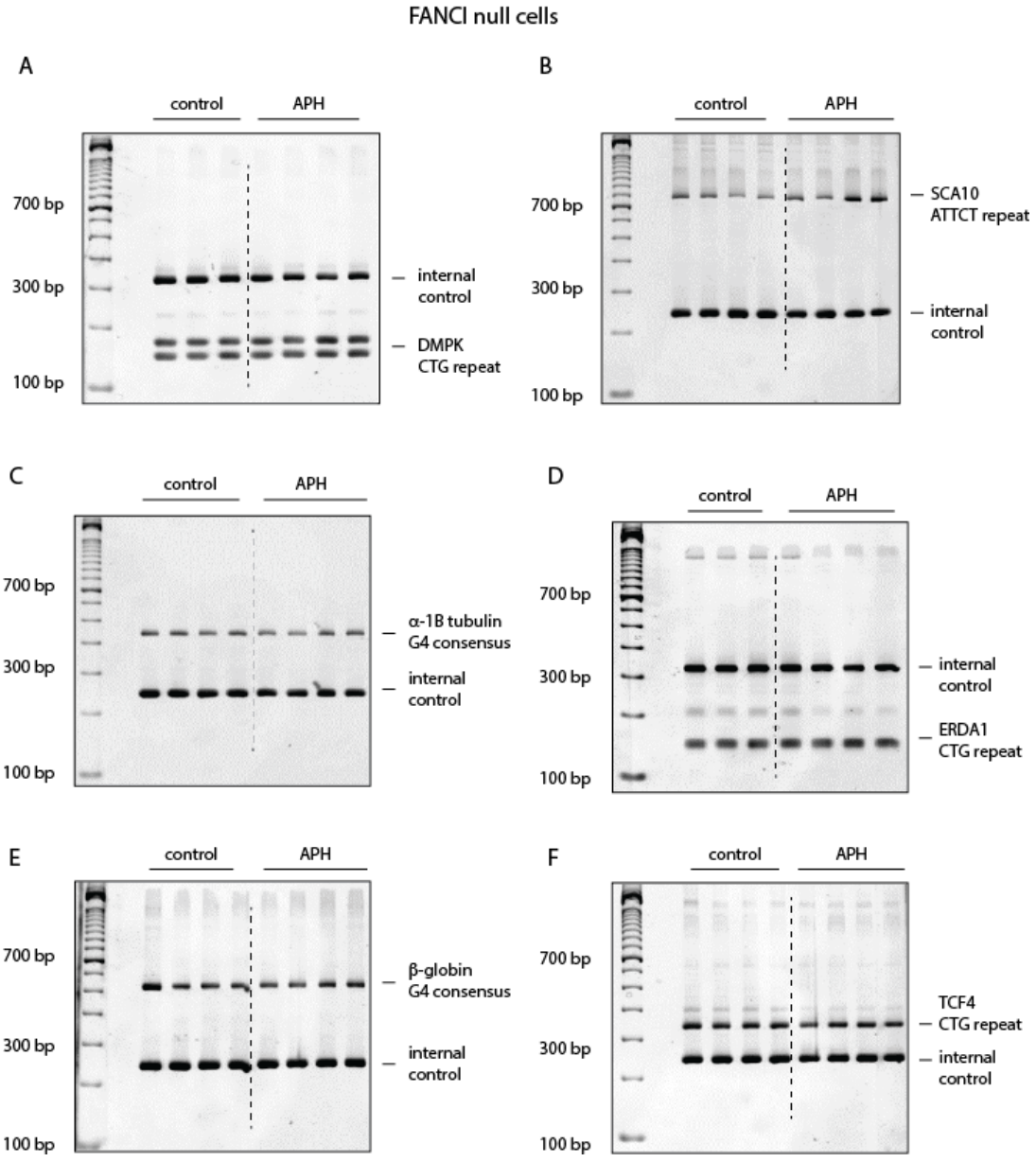

Figure 37. Aphidicolin treatment of FANCI null cells. Small pool PCR across repeated sequences in DNA from FANCI null patient fibroblast cells treated with aphidicolin.

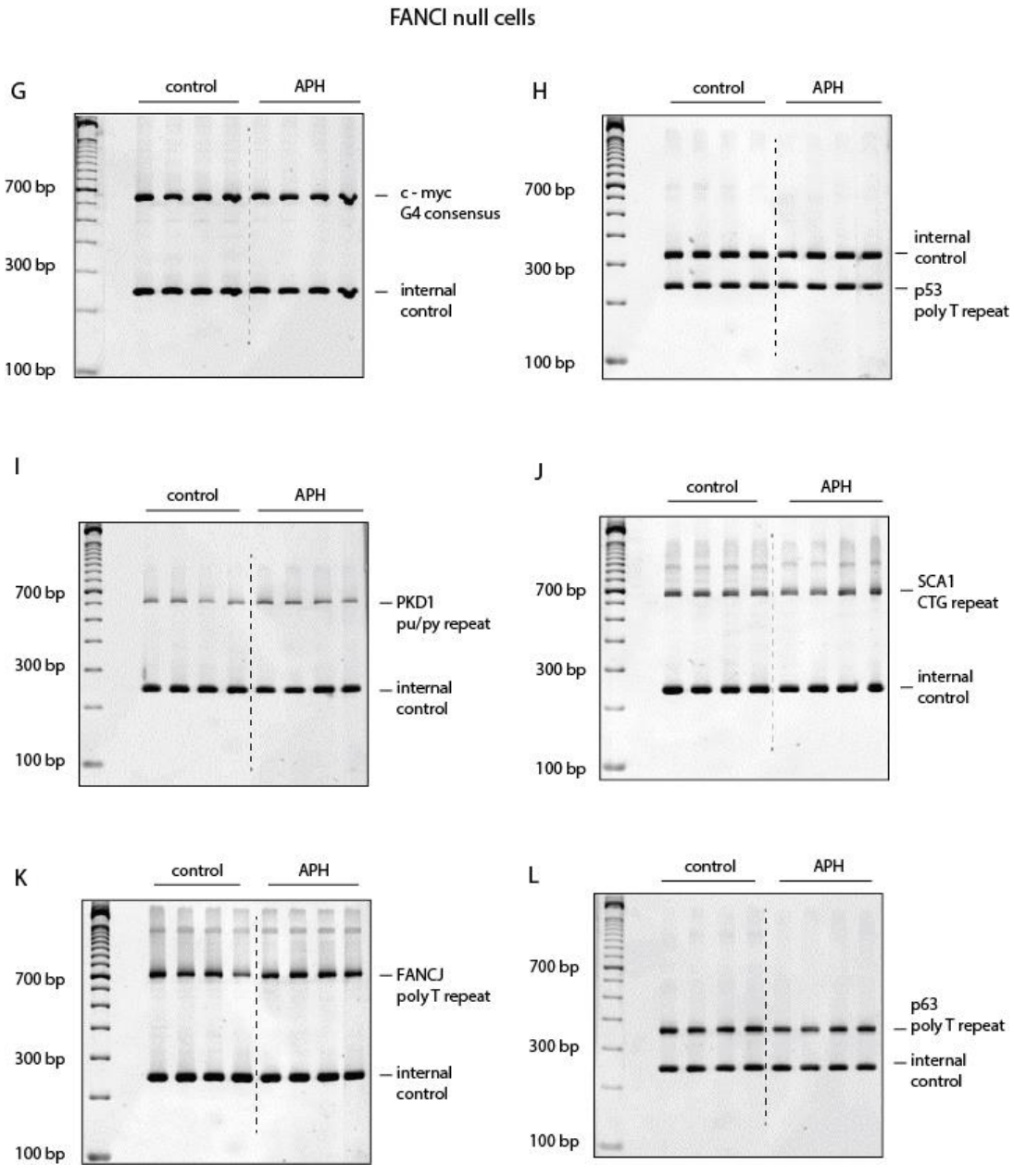

Figure 37 (continued). Aphidicolin treatment of FANCI null cells. Small pool PCR across repeated sequences in DNA from FANCI null patient fibroblast cells treated with aphidicolin.

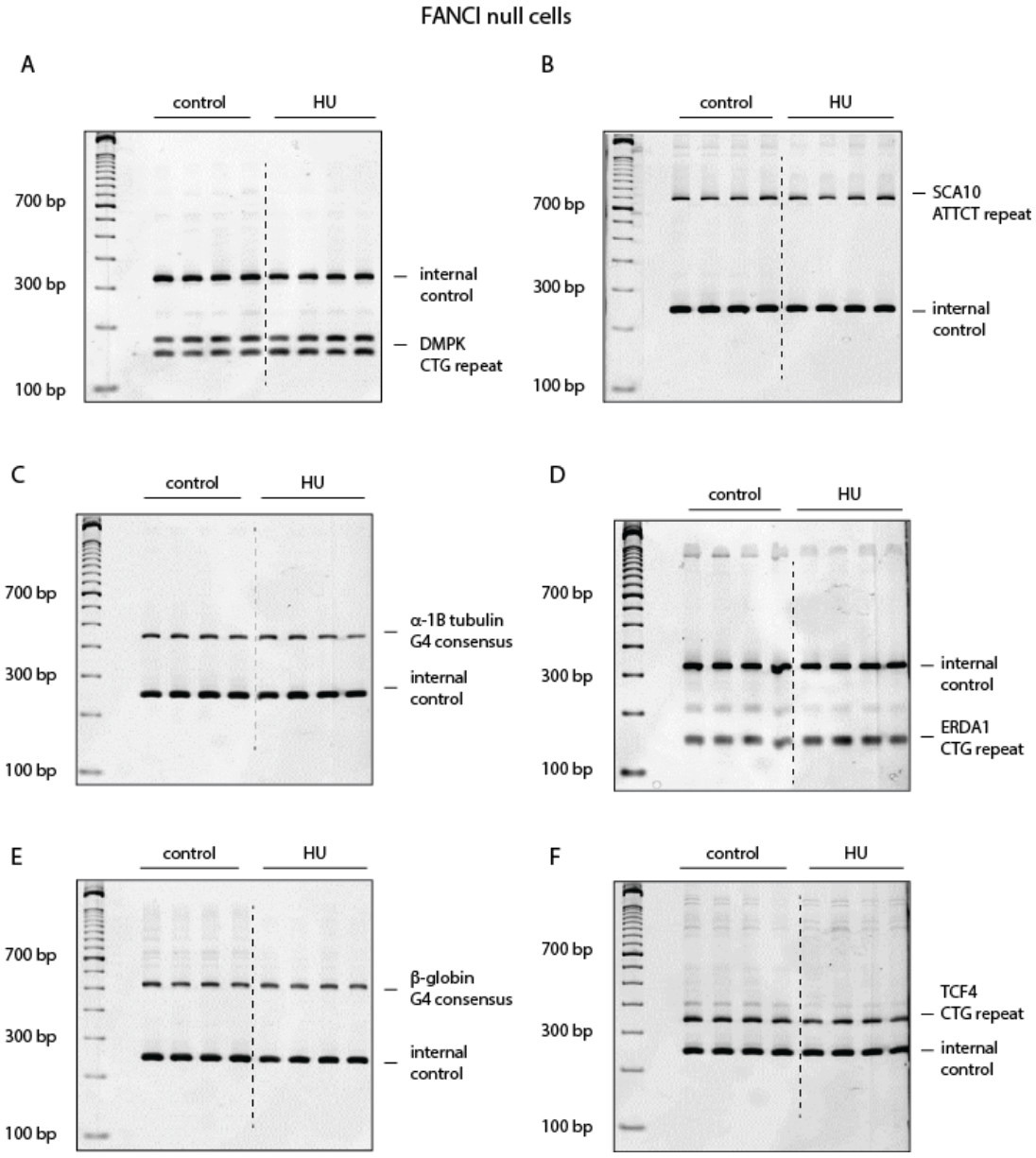

Figure 38. Hydroxyurea treatment of FANCI null cells. Small pool PCR across repeated sequences in DNA from FANCI null patient fibroblast cells treated with hydroxyurea.

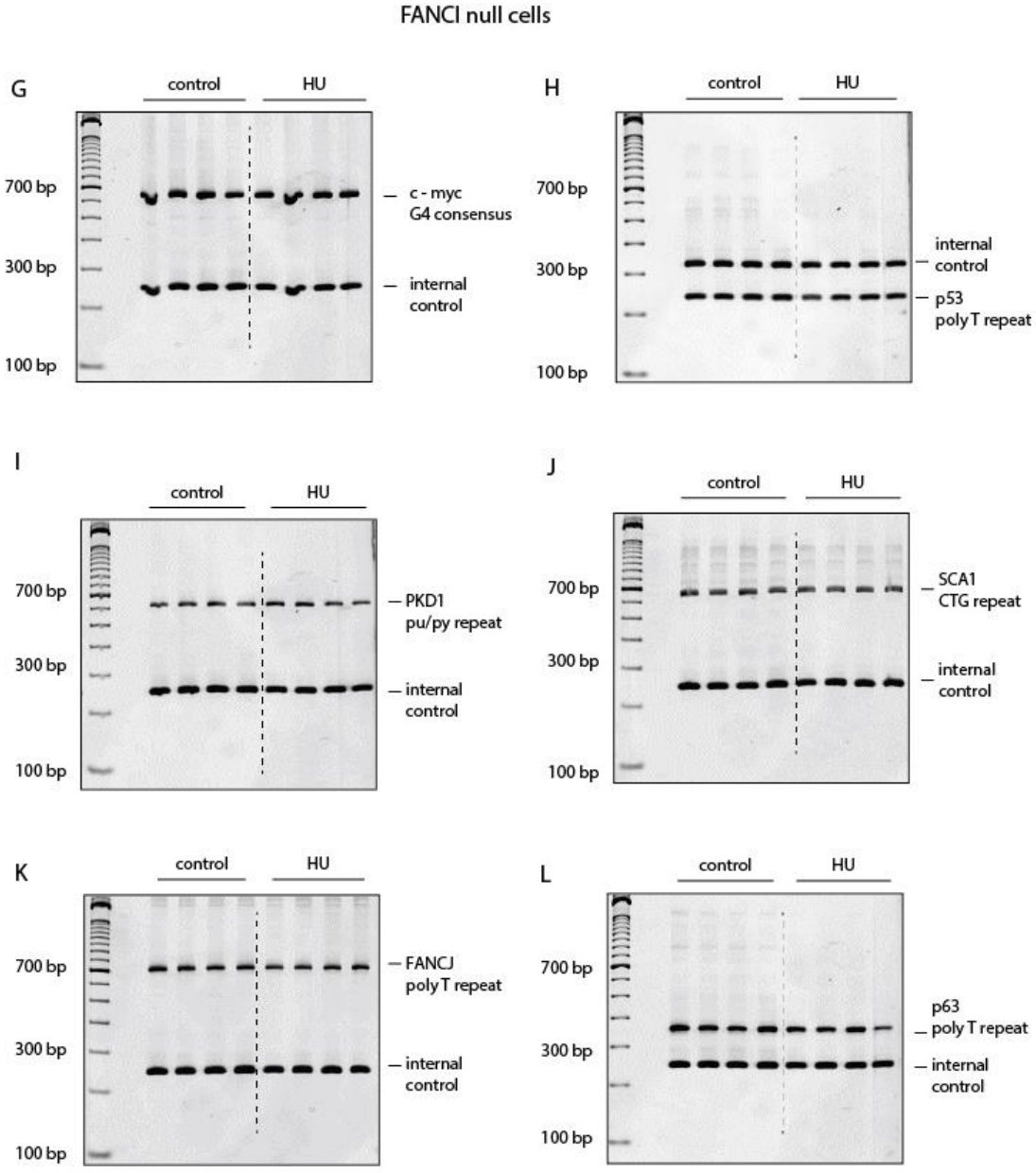

Figure 38 (continued). Hydroxyurea treatment of FANCI null cells. Small pool PCR across repeated sequences in DNA from FANCI null patient fibroblast cells treated with hydroxyurea.

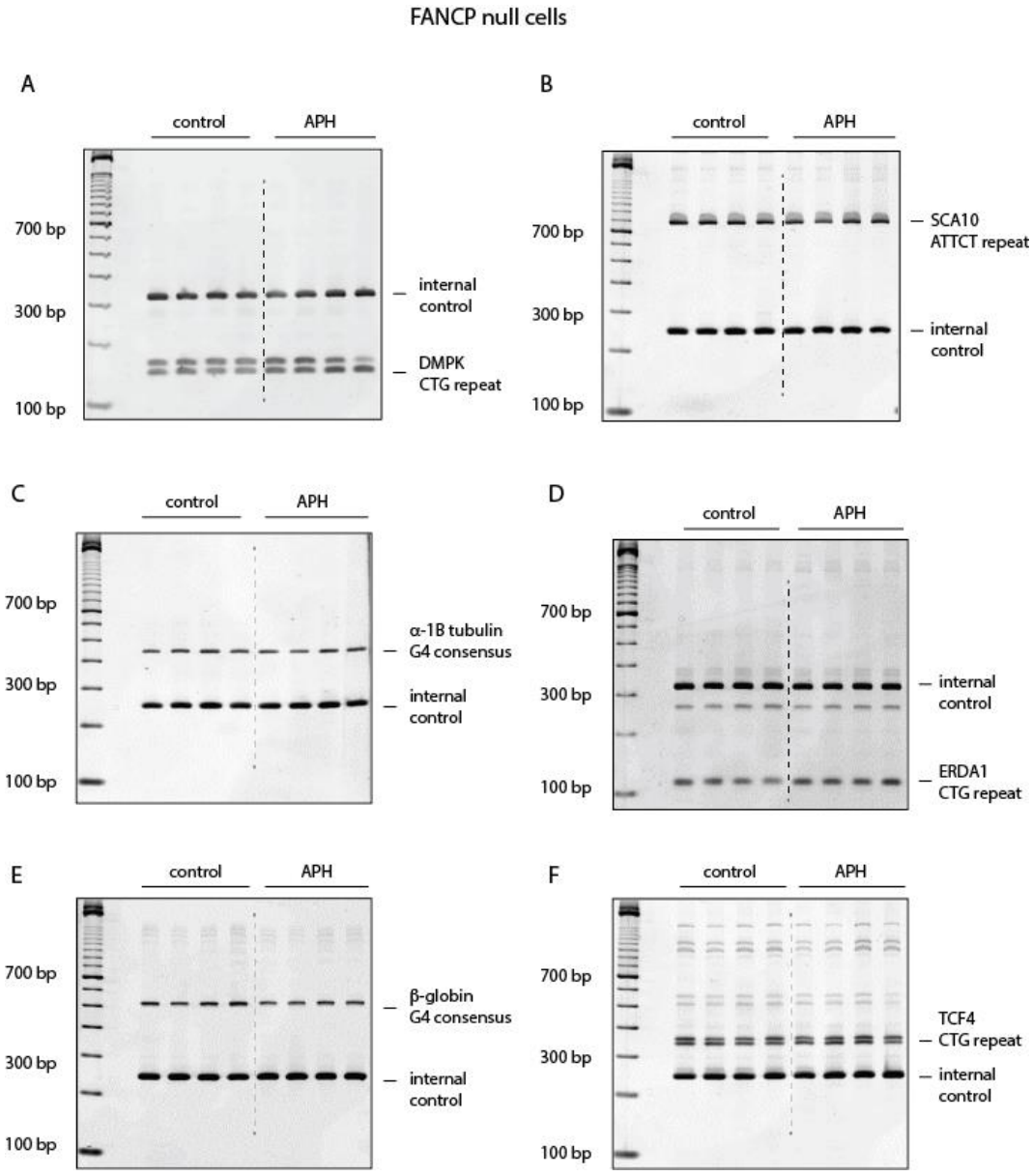

Figure 39. FANCP null cells do not show microsatellite signal loss treated with aphidicolin. Small pool PCR across repeated sequences in DNA from FANCP null patient fibroblast cells treated with aphidicolin.

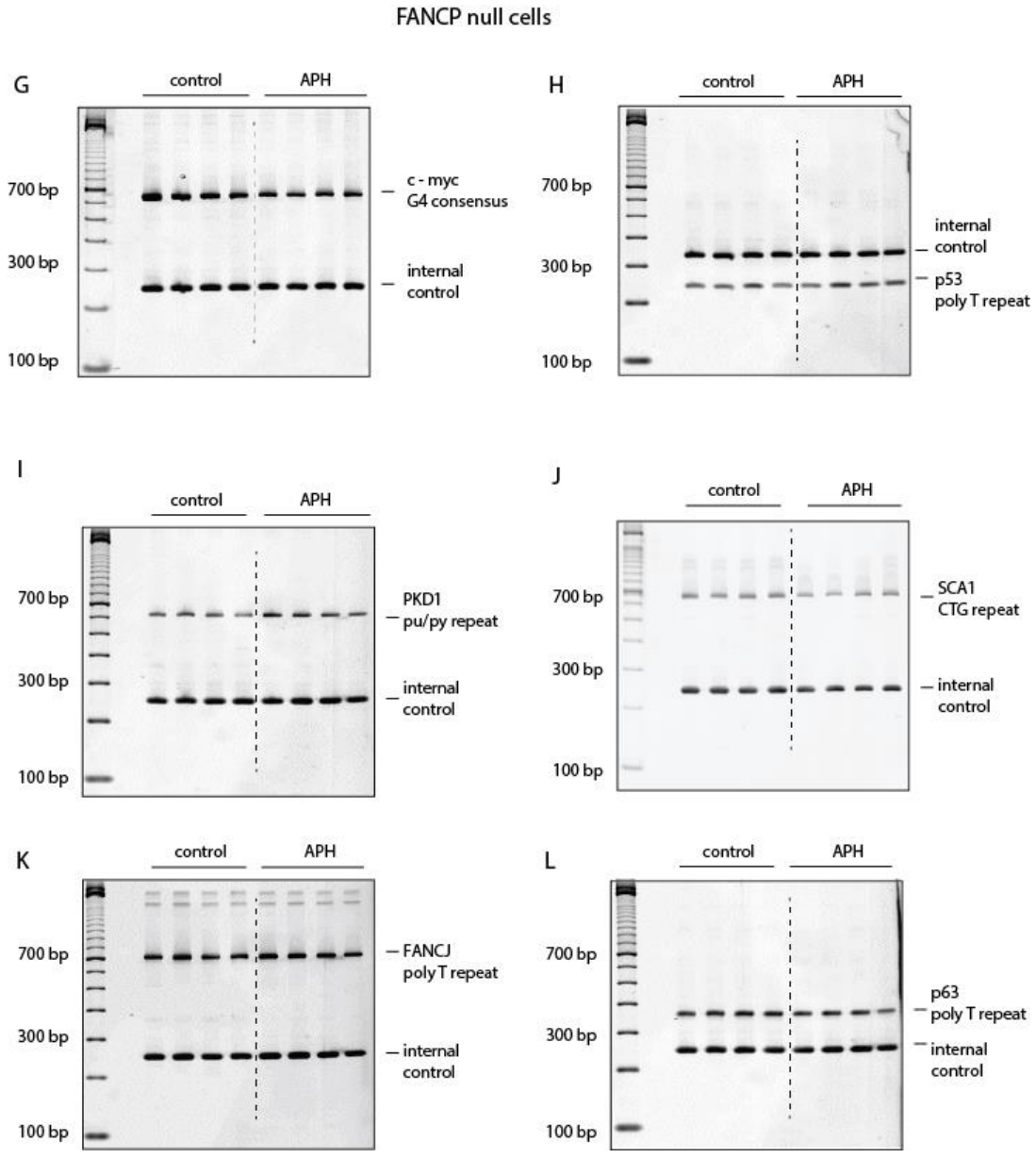

Figure 39 (continued). FANCP null cells do not show microsatellite signal loss treated with aphidicolin. Small pool PCR across repeated sequences in DNA from FANCP null patient fibroblast cells treated with aphidicolin.

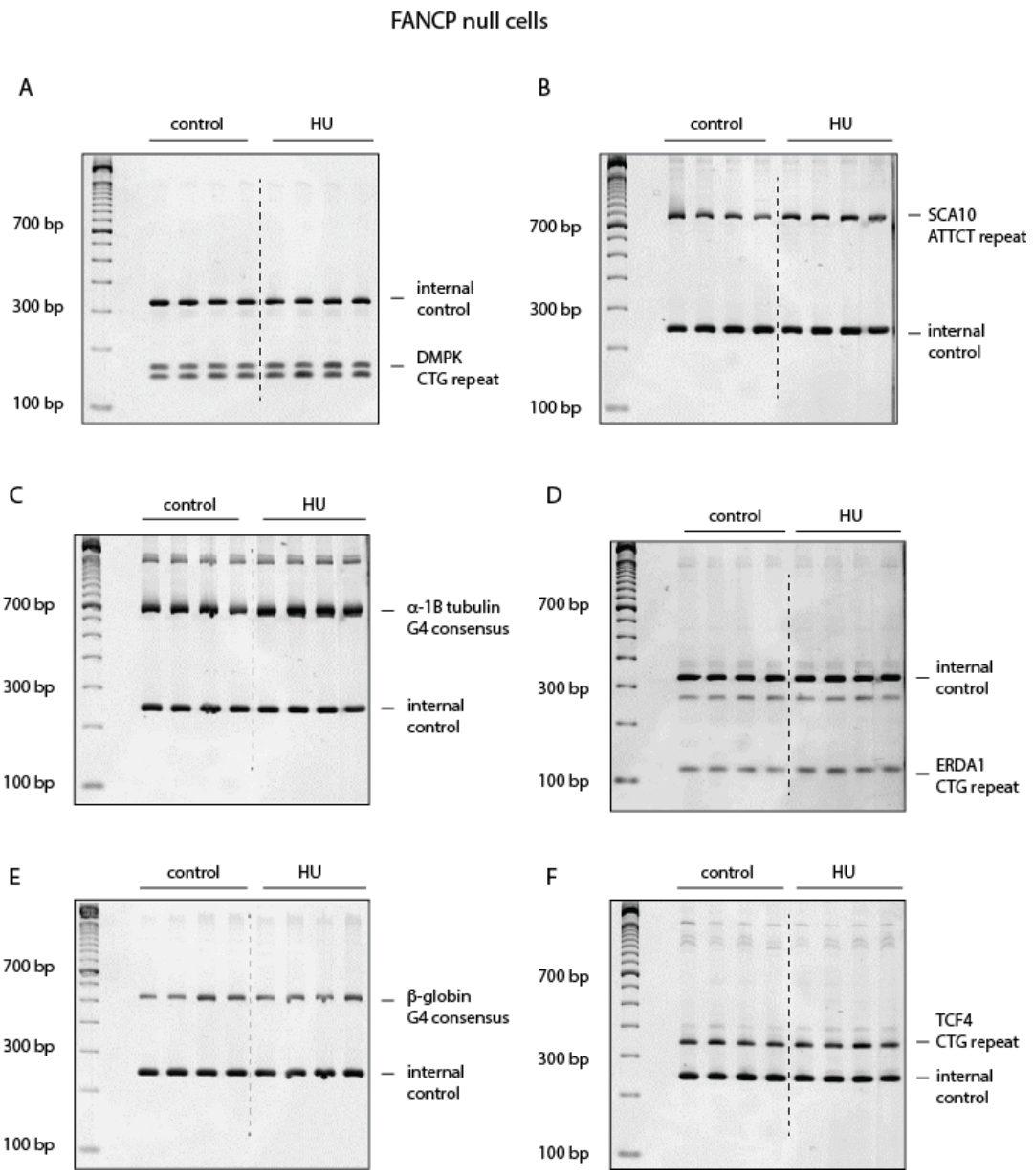

Figure 40. FANCP null cells do not show microsatellite signal loss treated with hydroxyurea. Small pool PCR across repeated sequences in DNA from FANCP null patient fibroblast cells treated with hydroxyurea.

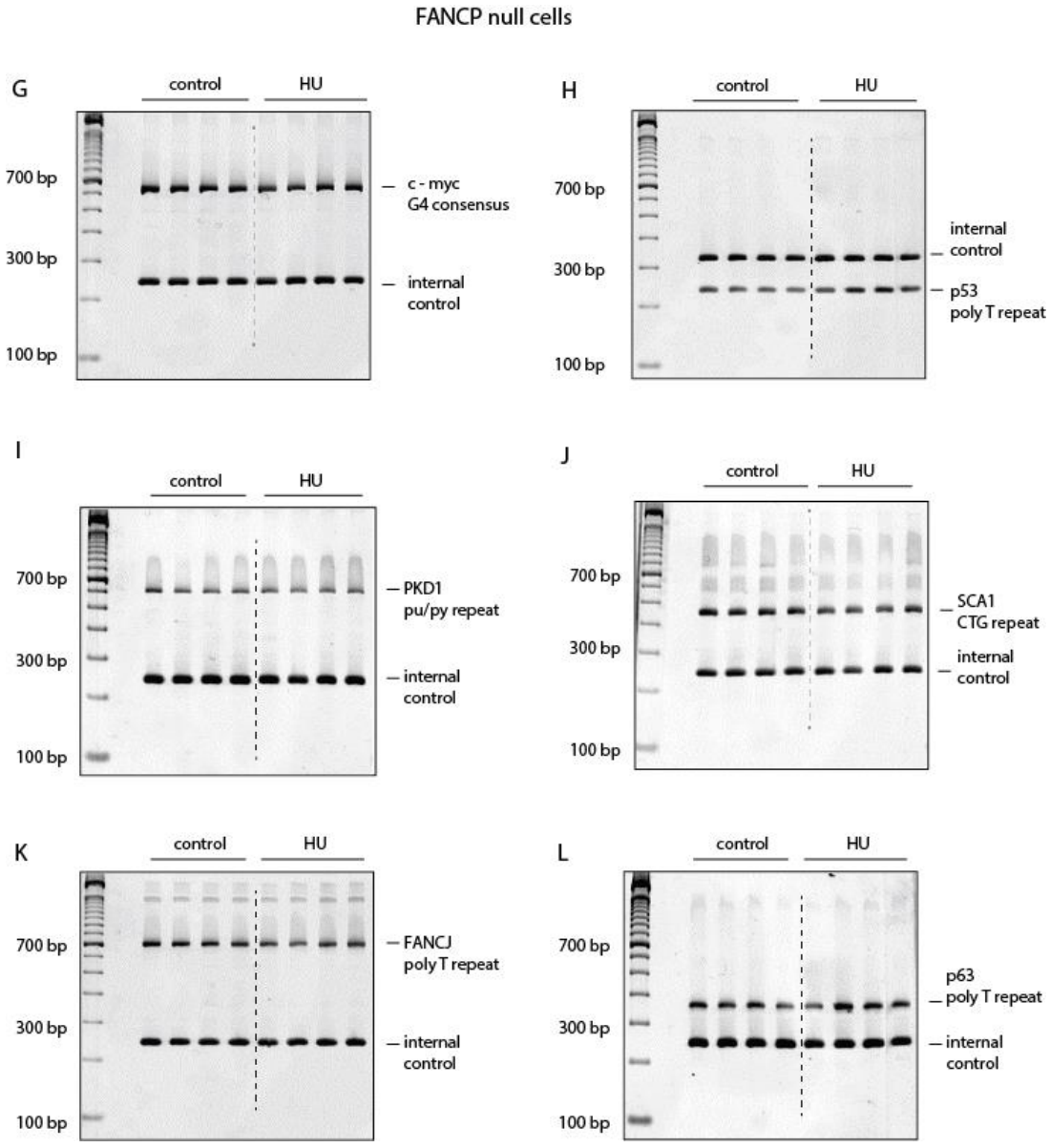

Figure 40 (continued). FANCP null cells do not show microsatellite signal loss treated with hydroxyurea. Small pool PCR across repeated sequences in DNA from FANCP null patient fibroblast cells treated with hydroxyurea.

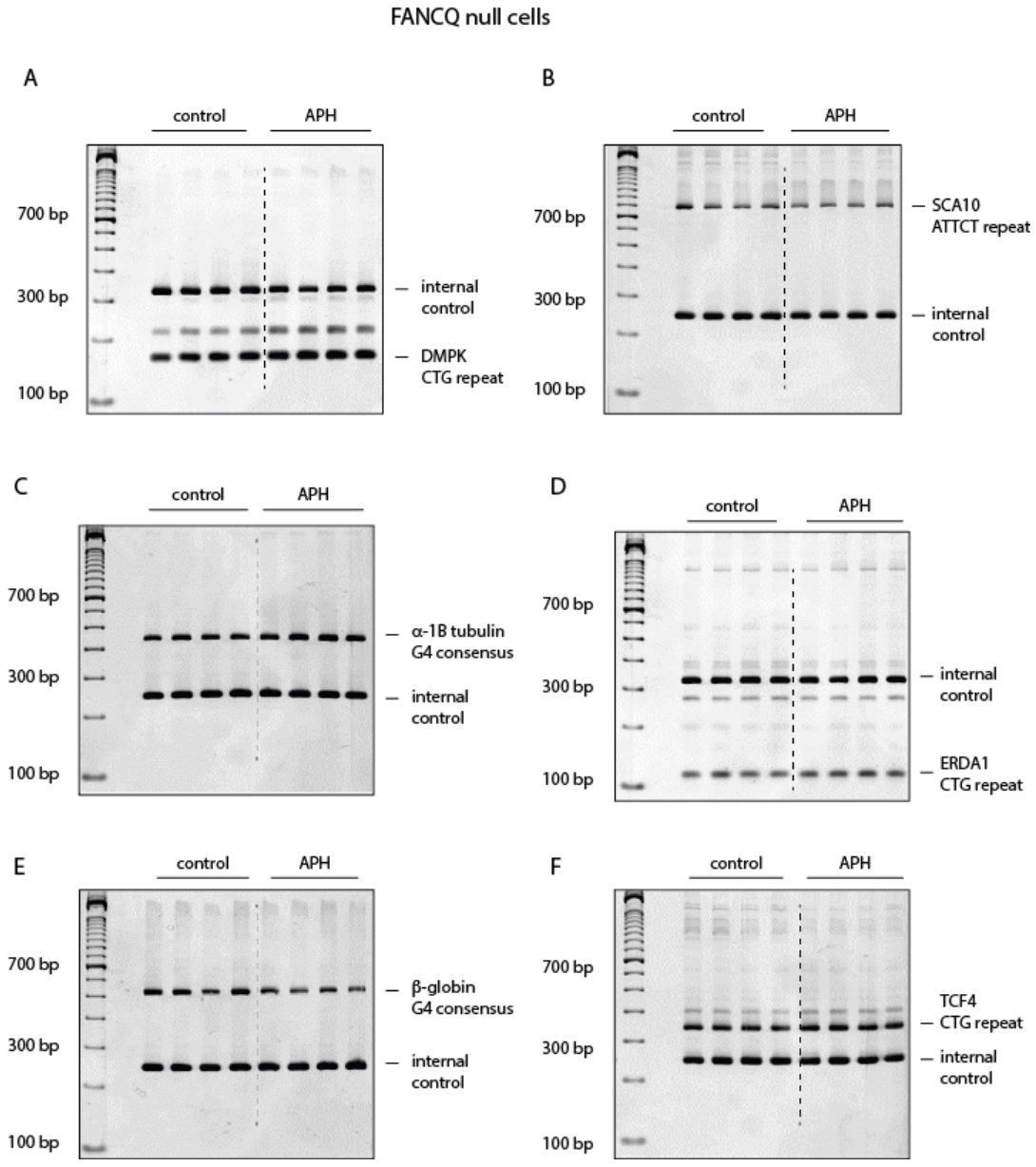

Figure 41. FANCQ null cells do not show microsatellite signal loss treated with aphidicolin. Small pool PCR results from FANCQ null patient fibroblast cells treated with aphidicolin.

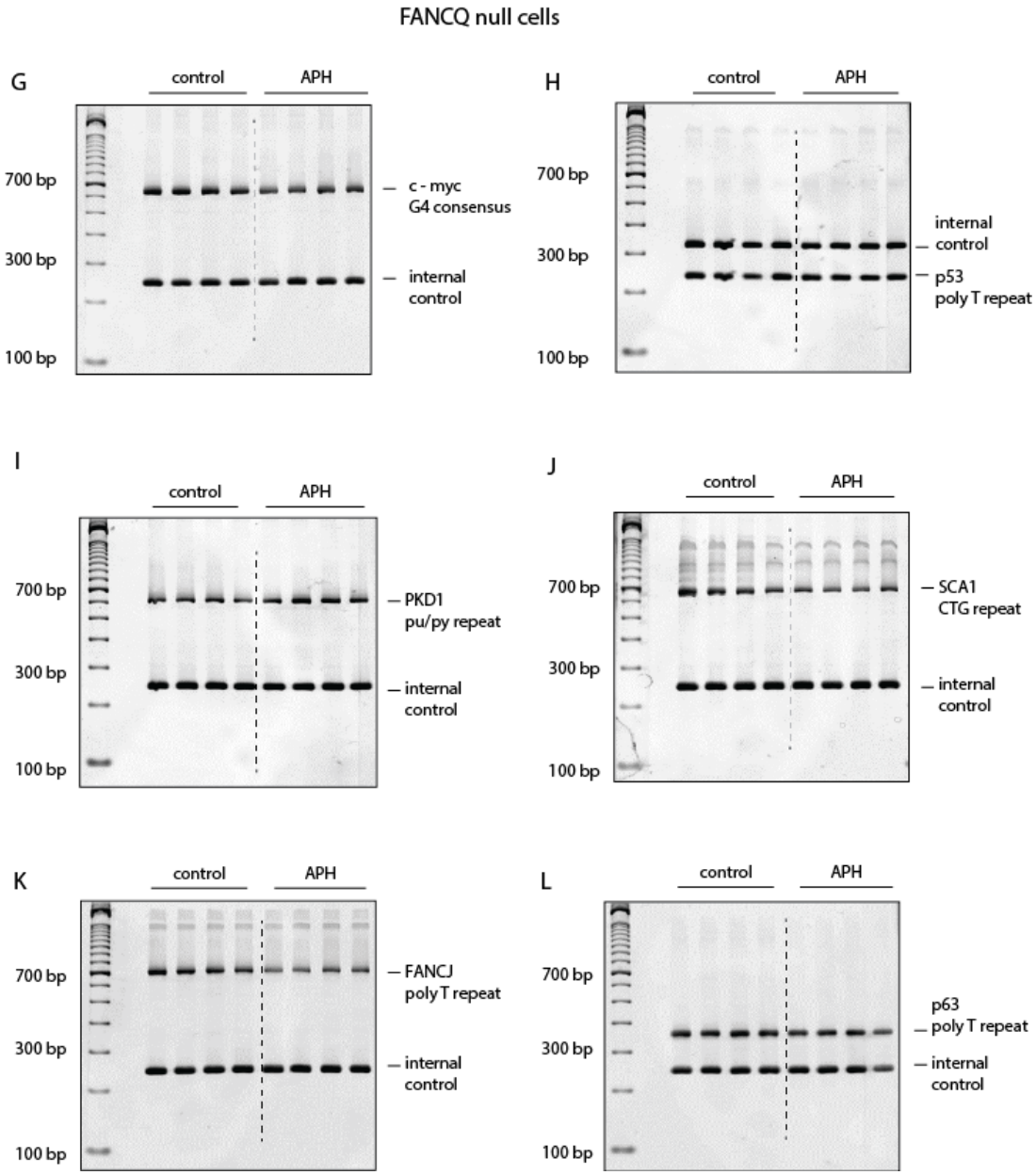

Figure 41 (continued). FANCQ null cells do not show microsatellite signal loss treated with aphidicolin. Small pool PCR results from FANCQ null patient fibroblast cells treated with aphidicolin.

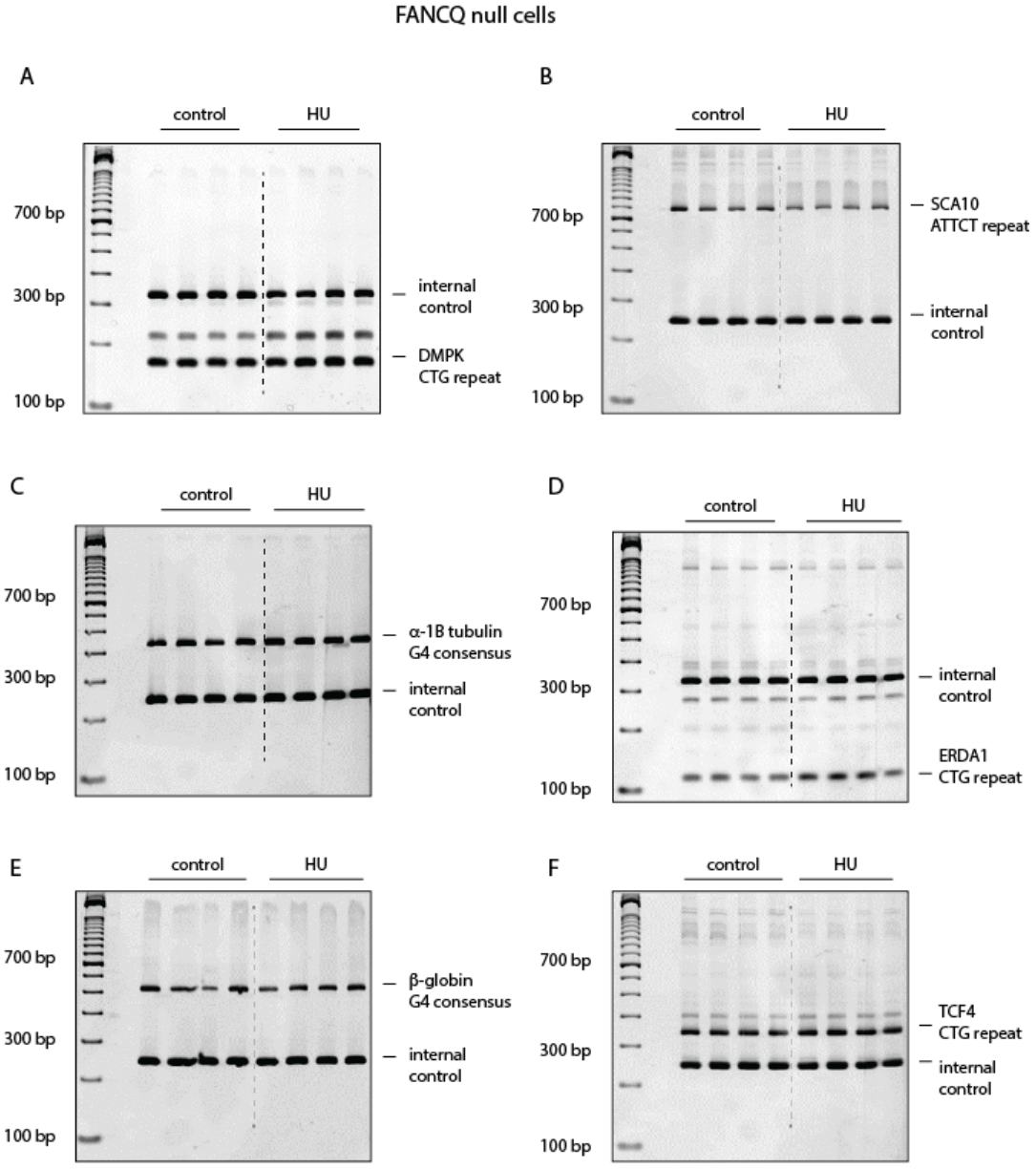

Figure 42. FANCQ null cells do not show microsatellite signal loss treated with hydroxyurea. Small pool PCR results from FANCQ null patient fibroblast cells treated with hydroxyurea.

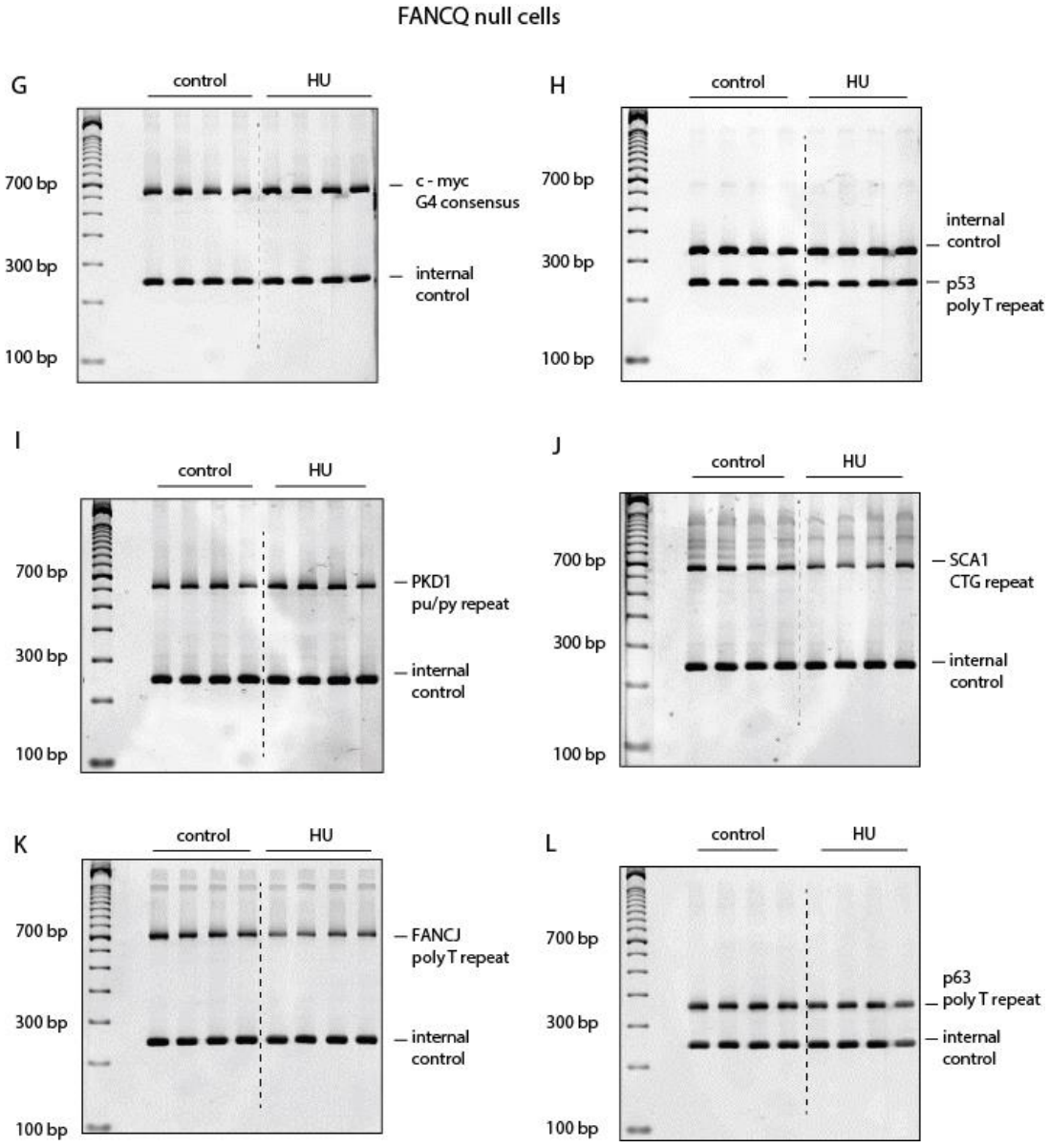

Figure 42 (continued). FANCQ null cells do not show microsatellite signal loss treated with hydroxyurea. Small pool PCR results from FANCQ null patient fibroblast cells treated with hydroxyurea.

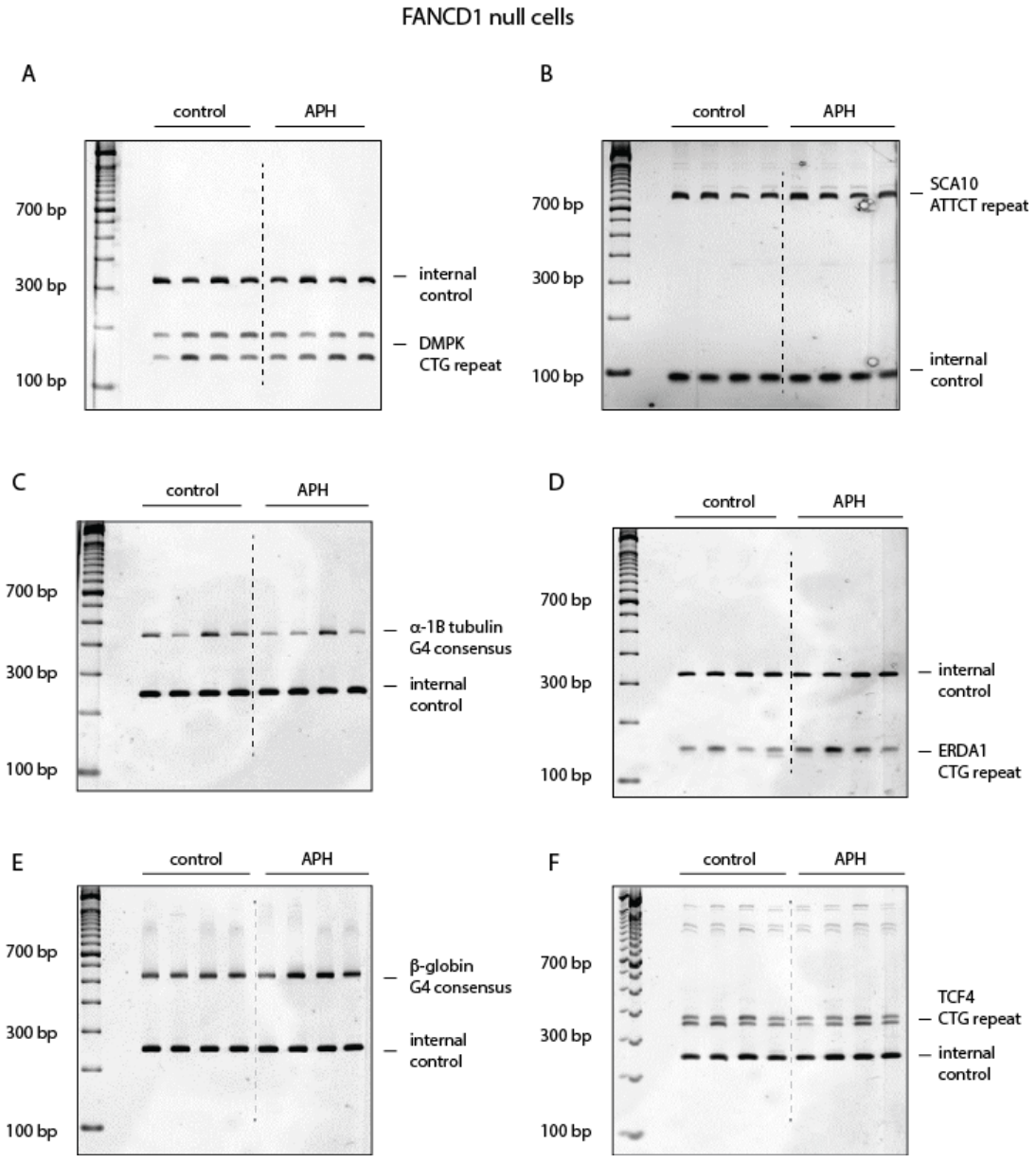

Figure 43. FANCD1 null cells do not show microsatellite signal loss treated with aphidicolin. Small pool PCR across repeated sequence loci from FANCD1 null patient fibroblast cells treated with aphidicolin.

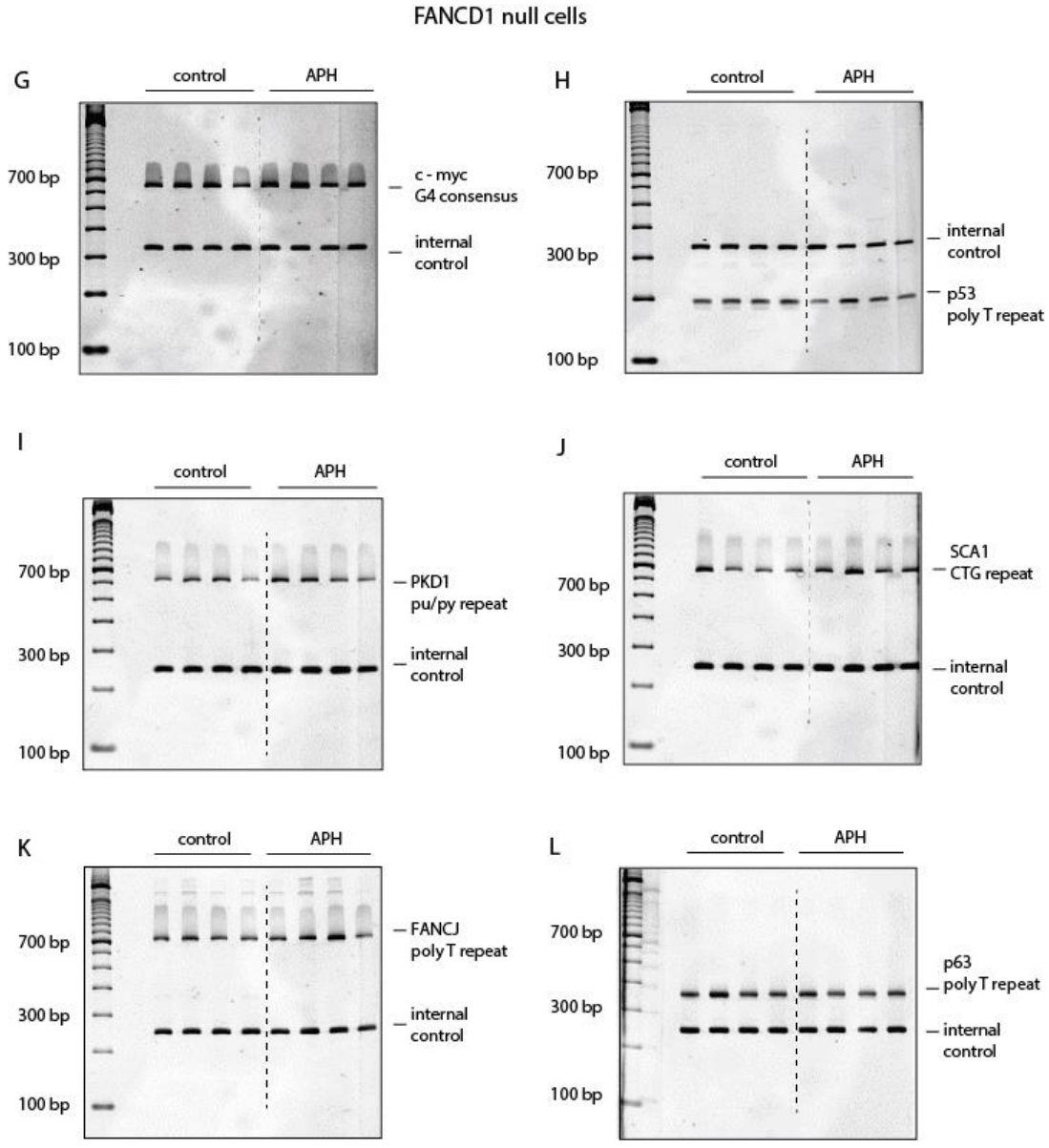

Figure 43 (continued). FANCD1 null cells do not show microsatellite signal loss treated with aphidicolin. Small pool PCR across repeated sequence loci from FANCD1 null patient fibroblast cells treated with aphidicolin.

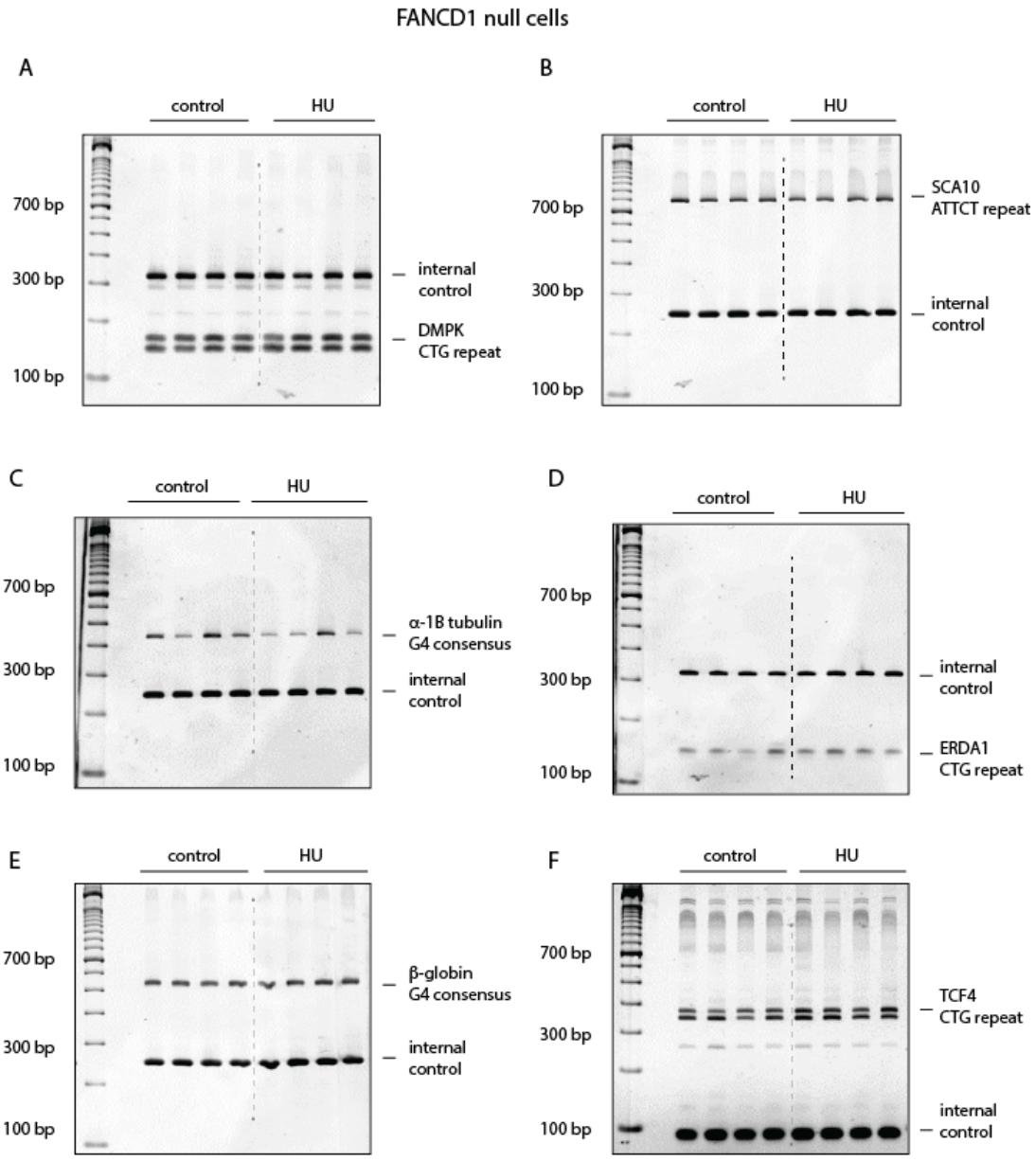

Figure 44. FANCD1 null cells do not show microsatellite signal loss treated with hydroxyurea. Small pool PCR across repeated sequence loci from FANCD1 null patient fibroblast cells treated with hydroxyurea.

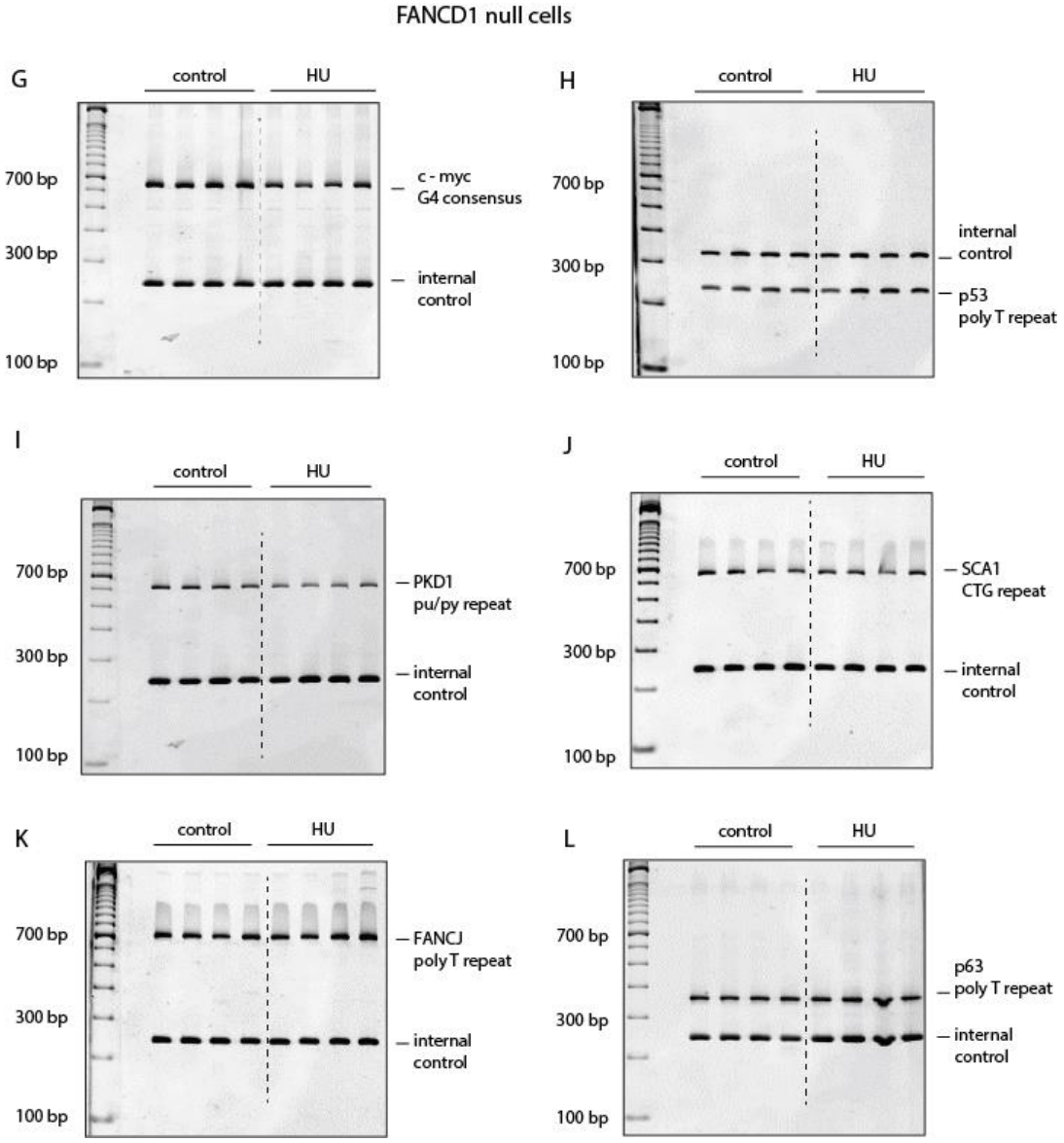

Figure 44 (continued). FANCD1 null cells do not show microsatellite signal loss treated with hydroxyurea. Small pool PCR across repeated sequence loci from FANCD1 null patient fibroblast cells treated with hydroxyurea.

Summary

Taken together, these results indicate microsatellite stabilization by FANCJ occurs independently of the activity of the other FA repair pathway proteins. We examined each step of the FA repair pathway (activation, core complex assembly, heterodimer formation and recruitment of downstream effectors) individually through the treatment of FANC null patient fibroblast cells with replicative stress (APH or HU). Results of these experiments confirmed the novel function of FANCJ in microsatellite stabilization occurs independently FA repair pathway function.

III. Loss of FANCJ with APH treatment leads to DSBs and subsequent translocations

Determination of sites along the genome recombined with the ectopic site (CTG)·(CAG) in the absence of FANCJ

The data presented above show a loss of a PCR signal when small pool PCR is performed with primers flanking the ectopic site (CTG)·(CAG) repeats in DM1 model cells treated with siRNA against FANCJ and aphidicolin or hydroxyurea (Figure 7 and 12). These data suggest that the PCR primer binding sites have been separated in cells depleted of FANCJ under replicative stress. However, sequences both upstream and downstream of the (CTG)·(CAG) repeats are still intact (Figure 8). Standard PCR primers were designed to obtain a PCR product extending from the known upstream site to the known downstream site across the (CTG)·(CAG) repeats (Figure 45A). DNA samples from $(CAG)_{102}$ (DM1 model) cells treated with both siRNA targeting FANCJ

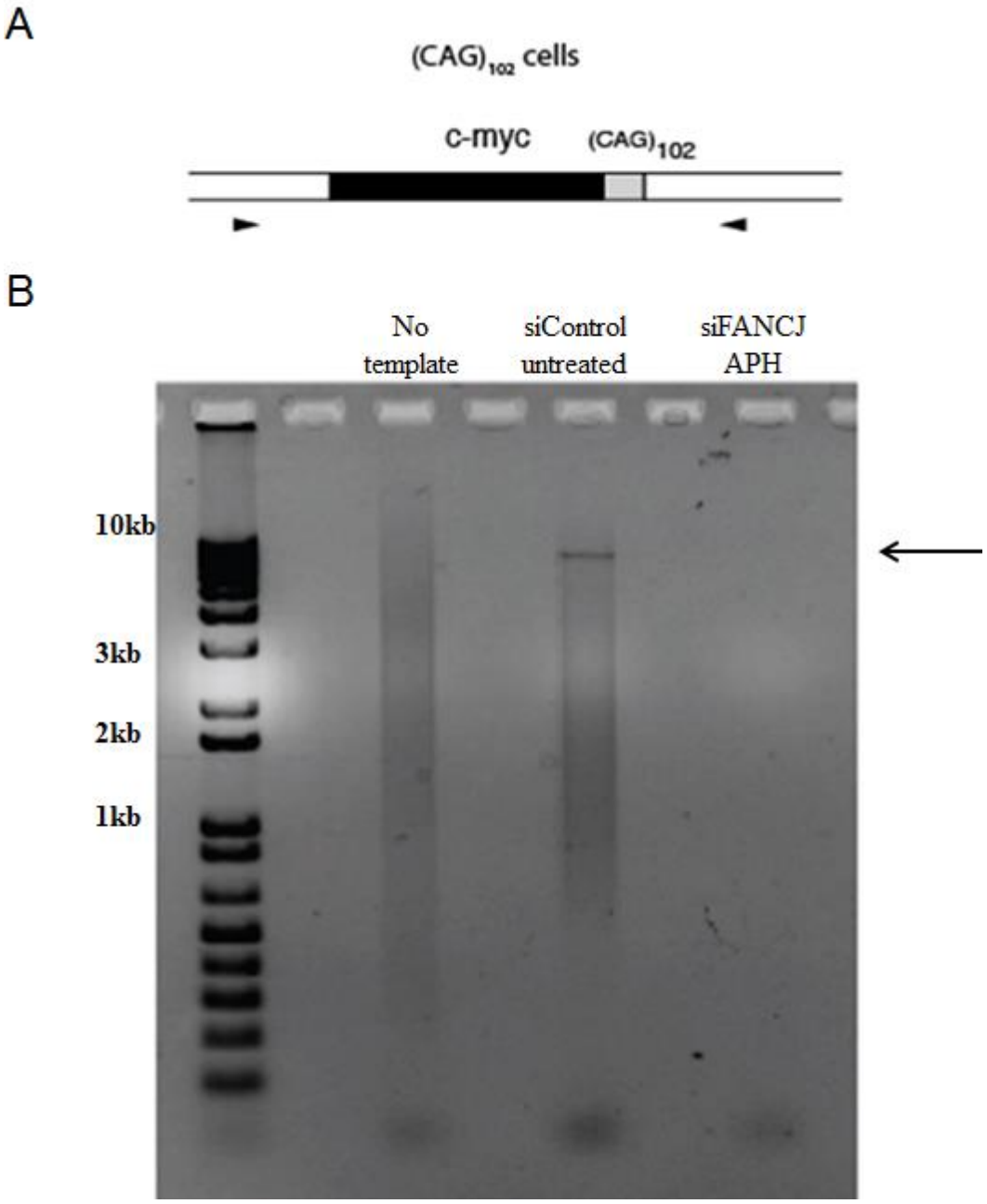

Figure 45: PCR of the ectopic site in DM1 model cells. **(A)** A map of the PCR primer set used to amplify from the known upstream sequence to the known downstream through the (CTG)·(CAG) repeats (arrowheads). **(B)** PCR results using primers shown in **(A)**.

and aphidicolin failed to produce a standard PCR product using these primers (Figure 45B) further confirming that the upstream and downstream sequences of the ectopic site are no longer within close proximity to one another. We believe a double strand break has occurred at or near the ectopic site (CTG)·(CAG) repeats (within 100bp flanking the repeat since the PCR primers used for spPCR bind 100bp upstream and downstream of the trinucleotide repeats produce loss of PCR signal) leading to recombination of the (CTG)·(CAG) repeats with unknown sites along the genome. To understand the mechanism by which PCR primer binding sites of the ectopic site are separated, we utilized a MseI endonuclease cut site (located near the ectopic site (CTG)·(CAG) repeats), a map of the ectopic site including MseI cut sites is shown in (Figure 46), coupled with intramolecular ligation to create circularized DNA containing known ectopic site sequence and unknown genomic DNA (Figure 46). To amplify only DNA sequences of interest, PCR amplification was performed using inverse PCR (iPCR) primers (Figure 46) targeting ectopic site sequences still present after treatment with both siRNA against FANCJ and aphidicolin. PCR products were separated on an agarose gel; a representative image is shown in Figure 47B. PCR products appeared as a smear on the agarose gel (Figure 47B) indicating multiple sites along the genome recombined with the ectopic (CTG)·(CAG) repeats, size variations (leading to multiple bands on the agarose gel "smear") occur based upon the closest MseI recognition sequence at the unknown genomic site. If recombination had not occurred, a single band would be present on the gel, representative of the distance between both MseI sites flanking the (CTG)·(CAG) repeats of the ectopic site (as seen in control DNA digested with MseI and circularized (Figure 47B). We used next generation sequencing (NGS) to create

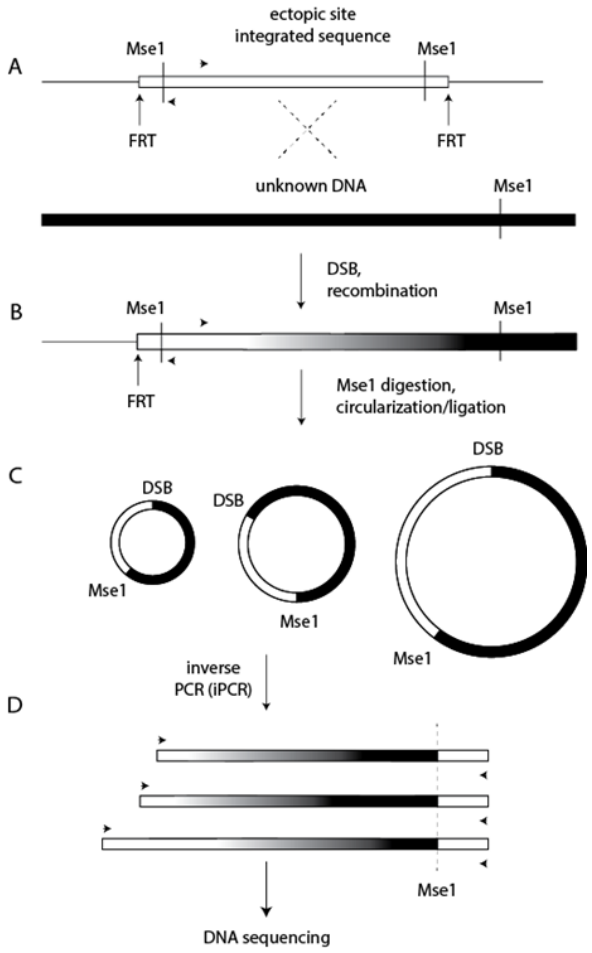

Figure 46: Schematic of experimental design for inverse PCR. **(A)** A map of the ectopic site (white bar) recombining with an unknown sequence in the genome (black bar). **(B)** A schematic of the translocation byproduct containing a section of known ectopic site sequence and unknown genomic sequence. Also denoted are MseI cut sites (known at the ectopic site and approximated in the unknown sequence). **(C)** Circular DNA products after MseI digestion and ligation. **(D)** Amplification of circularized genomic DNA with reverse PCR primers (arrowheads in **(B)**). Inverse PCR products containing known ectopic site sequence and unknown genomic sequence, these PCR products were used for next generation sequencing.

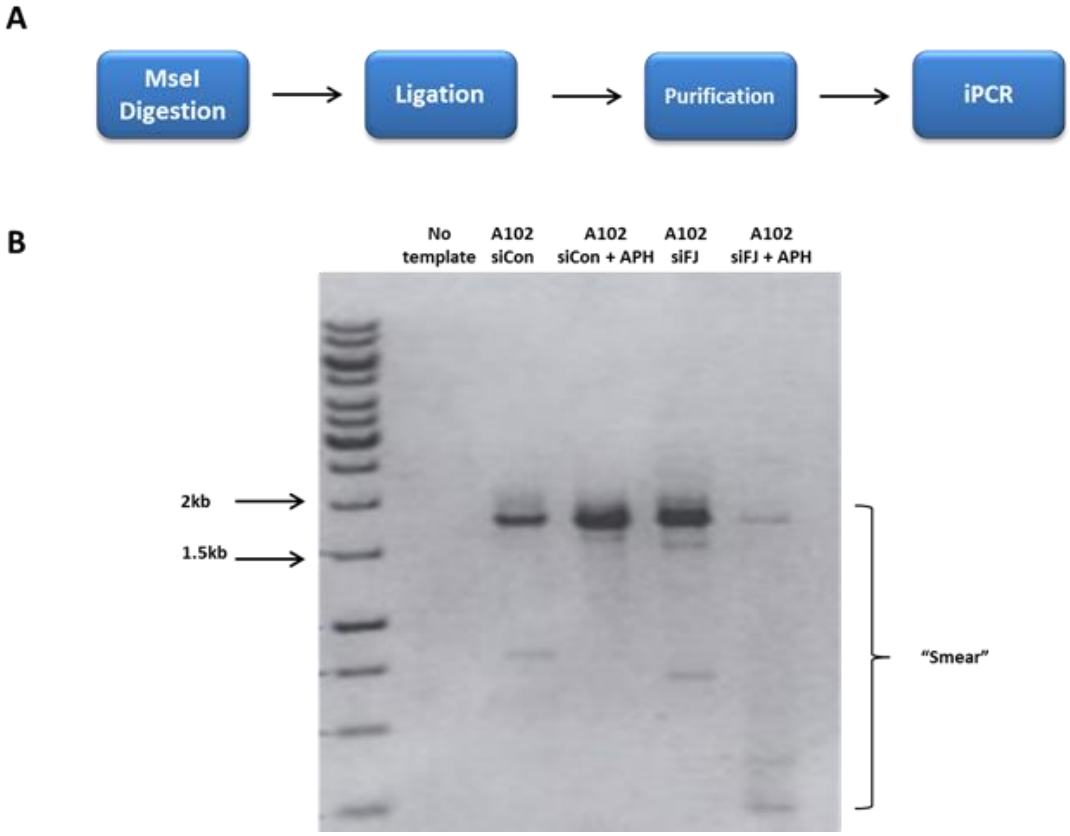

Figure 47: iPCR of CAG_{102} cells. **(A)** A map of the approach to produce inverse PCR product. **(B)** Represent agarose gel of inverse PCR products.

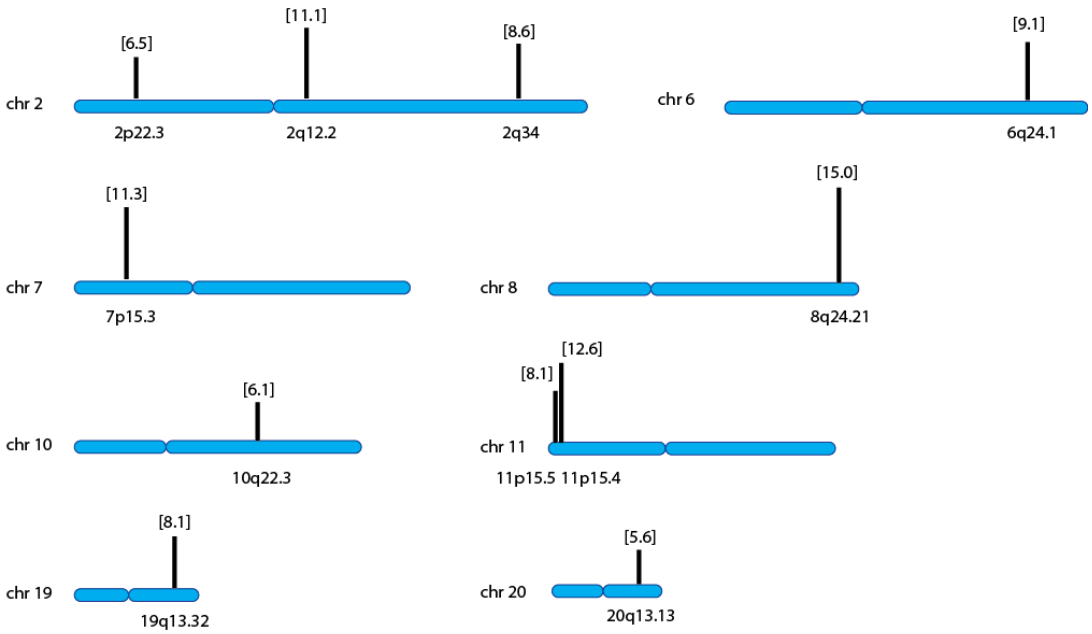

Figure 48. Translocation sites identified by next generation sequencing. Cytogenetic locations of DNA sequencing reads containing translocation junctions. [bars represent $\log_2$(read copy number)]

Chromosome	Cytogenetic Position	Microsatellite
Chr2	p22.3	G300
Chr2	q12.2	G4
Chr2	q34	G4
Chr7	p15.3	G4
Chr8	q24.21	G4
Chr11	p15.4	G4
Chr20	q13.13	G4
Chr6	q24.1	T20
Chr19	13.32	(CTG)20

Table 2. Microsatellite sequences associated with translocation sites. Majority of the translocation sites identified by next generation sequencing had a microsatellite present in close proximity.

Chromosome	Cytogenetic Position	Disease Asscioation
Chr2	p22.3	small cell lung carcinoma; sensorineural nonsyndromic recessive deafness; Maffucci syndrome; corticobasal degeneration
Chr2	q12.2	small cell lung carcinoma
Chr2	q34	breast cancer
Chr6	q24.1	breast cancer, neuroblastoma, melanoma; ovarian cancer
Chr7	p15.3	ovarian cancer; Beckwith Wiedemann syndrome; Dandy Walker syndrome; hand-foot-genital syndrome; myeloma
Chr8	q24.21	leukemia; lymphoma; gliomas; renal cell carcinoma; male breast cancer
Chr10	q22.3	non-small cell lung cancer; prostate cancer; Cowden disease; breast cancer; ovarian cancer
Chr11	p15.4-5	Silver-Russell syndrome; Beckwith Wiedemann syndrome; T lymphoid leukemia; breast cancer
Chr20	q13.13	hepatocellular carcinoma; colorectal cancer

Table 3. Cytogenetic sites are associated with cancer and developmental disorders. All cytogenetic positions mapped to the translocation sites are associated with cancer or developmental disease.

a PCR amplicon library and determine which sites along the genome recombined near the ectopic (CTG)·(CAG) repeats at the ectopic site in $(CAG)_{102}$ cells treated with FANCJ siRNA and aphidicolin.

NGS data confirmed translocations had occurred between the ectopic site and unknown genomic sites. Approximately 28,000 of the reads from the sequencer contained translocation junctions between the ectopic site and other chromosomes (Figure 48). These translocations appear to have occurred nonrandomly. A large majority of translocations occurred between the ectopic site located on chromosome 18 and the endogenous c-myc locus on chromosome 8q24.21 via nonallelic homologous recombination (NAHR). In addition, recombination occurred with the endogenous $(CTG)_{20}$ of the DMPK locus (chromosome 19q13.32) further suggesting the ectopic site is a substrate for NAHR. Microsatellite sequences were observed in close proximity to the translocation junction of the identified chromosome sites further supporting translocations occur via nonrandom recombination (Table 2). Although, translocation sites without microhomology to the ectopic site were also observed. Lastly, the cytogenetic positions of the translocation sites have previously been associated with a wide array of the cancers, developmental disorders, and developmental disorders with predisposition to cancer (Table 3).

Summary

The data presented focuses on understanding the mechanisms that lead to loss of PCR signal in cells depleted of FANCJ under replicative stress. We utilized an iPCR approach to amplify MseI digested genomic DNA containing sequences of the ectopic site present after treatment with FANCJ siRNA and aphidicolin. Next generation

sequencing identified genomic chromosomal sites fused with the (CTG)·(CAG) ectopic site sequences. We conclude that replication stress induces double strand breaks resulting in nonrandom chromosome translocations in FANCJ depleted cells.

IV. FANCJ stabilizes microsatellites independently of BLM/WRN function

In addition to Fanconi anemia, several other genome instability associated diseases exist including Bloom's Syndrome and Werner's Syndrome. Bloom's Syndrome (BS) is an autosomal recessive disease derived from mutations in the BLM (Bloom) helicase (German, 1995). Similarly, Werner's Syndrome (WS), also an autosomal recessive disease, results from mutations within the WRN (Werner) helicase (Holliday et al., 1974). BLM helicase functions to unwind noncanonical DNA structures to protect against sister chromatid exchanges and subsequent DNA damage (Li et al., 2001; Mohaghegh et al., 2001). Recent evidence suggests that BLM works in conjunction with the FA repair pathway to resolve replication stress and maintain chromosome stability (Deans and West, 2009; Suhasini and Brosh, 2012; Suhasini et al., 2011). Moreover, FANCM, the initiator protein of the FA repair pathway contains two separate binding domains: one for FA repair pathway activation and a second for the recruitment of the BLM complex (Deans and West, 2009). More recently, it was shown that BLM helicase and FANCJ directly interact and are functionally linked (Suhasini et al., 2011). BLM unwinds DNA in a 3' to 5' orientation and FANCJ helicase unwinds DNA in a 5' to 3' direction (Li et al., 2001; Suhasini et al., 2011). Additionally, a direct relationship between protein level of BLM helicase and FANCJ helicase exists, BLM helicase protein

levels are decreased in FANCJ helicase depleted cells (Suhasini et al., 2011). Evidence exists suggesting that BLM helicase and FANCJ helicase work in concert to maintain genome stability by unwinding DNA in opposite orientations (Suhasini et al., 2011). In comparison, WRN helicase also unwinds noncanonical DNA structures to protect telomeres and prevent pre-mature aging, a hallmark of WS (Li et al., 2001; Mohaghegh et al., 2001). Current research implies a role for WRN helicase in the stability of fragile sites as evidenced by the accumulation of gaps and breaks in DNA at common fragile sites after aphidicolin treatment of WS (WRN null) patient fibroblasts compared to control normal fibroblast cells treated with aphidicolin (Pirzio et al., 2008). Additionally, *in vitro* work with human nuclear extracts and (CTG)·(CAG) containing plasmids showed WRN is necessary for resolving large CTG/CTG hairpins (Chan et al., 2012). Most recently, WRN has been shown to compensate for loss of the Fanconi anemia repair pathway in the protection against genome instability after mitomycin C (MMC) treatment (Aggarwal et al., 2013). Our data indicates a role for FANCJ in the protection of microsatellite stability; thus it is possible that interactions between BLM/WRN and FANCJ may also be necessary for the stability of trinucleotide repeats. A model for collaboration between FANCJ and BLM/WRN to maintain genome stability is depicted in Figure 49.

FANCJ can function independently of BLM helicase activity

To understand the role BLM helicase may have in conjunction with FANCJ to maintain microsatellite stability, we knocked down BLM helicase using a pool of four siRNAs in CTG_{102}·CAG_{102} cells (Figure 50B) and exposed them to the replicative stressor aphidicolin. Cells depleted of BLM and treated with APH did not show

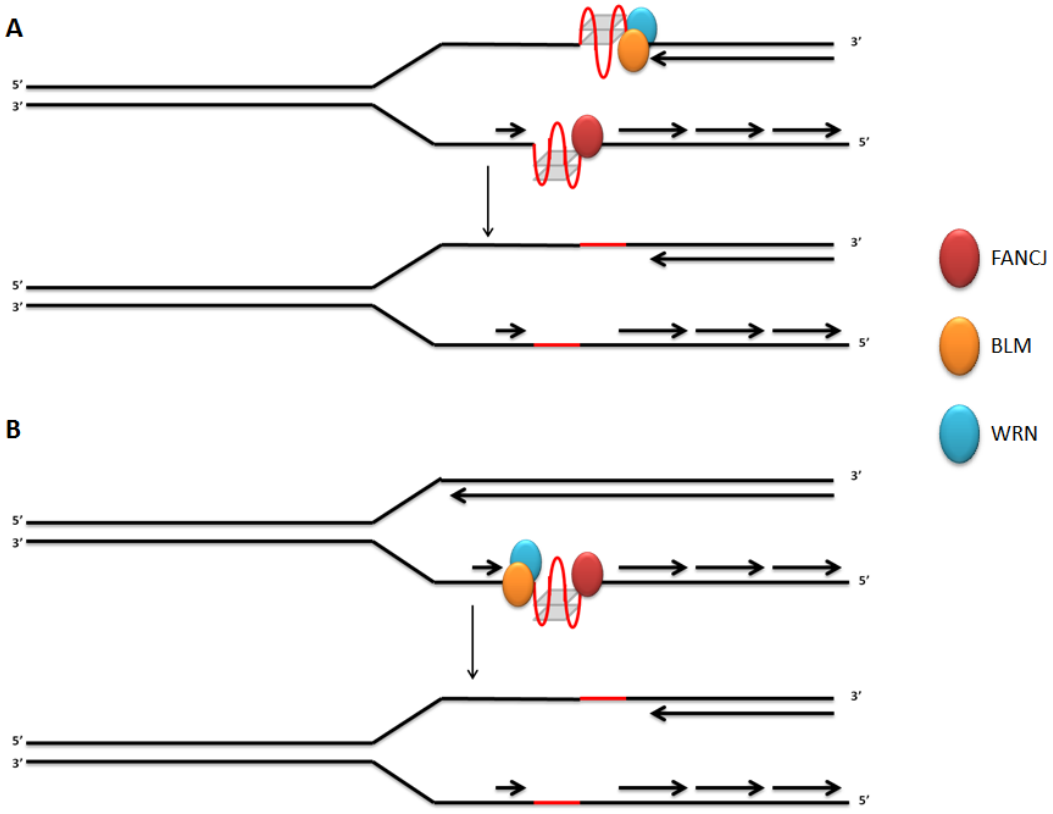

Figure 49: FANCJ works in conjunction with WRN or BLM to unwind secondary DNA structures. A proposed model for FANCJ to collaborate with WRN or BLM helicase to unwind non-B form structures simultaneously on the leading template (WRN or BLM) and lagging template (FANCJ) **(A)** or the same noncanonical DNA structure **(B)** for error-free DNA replication.

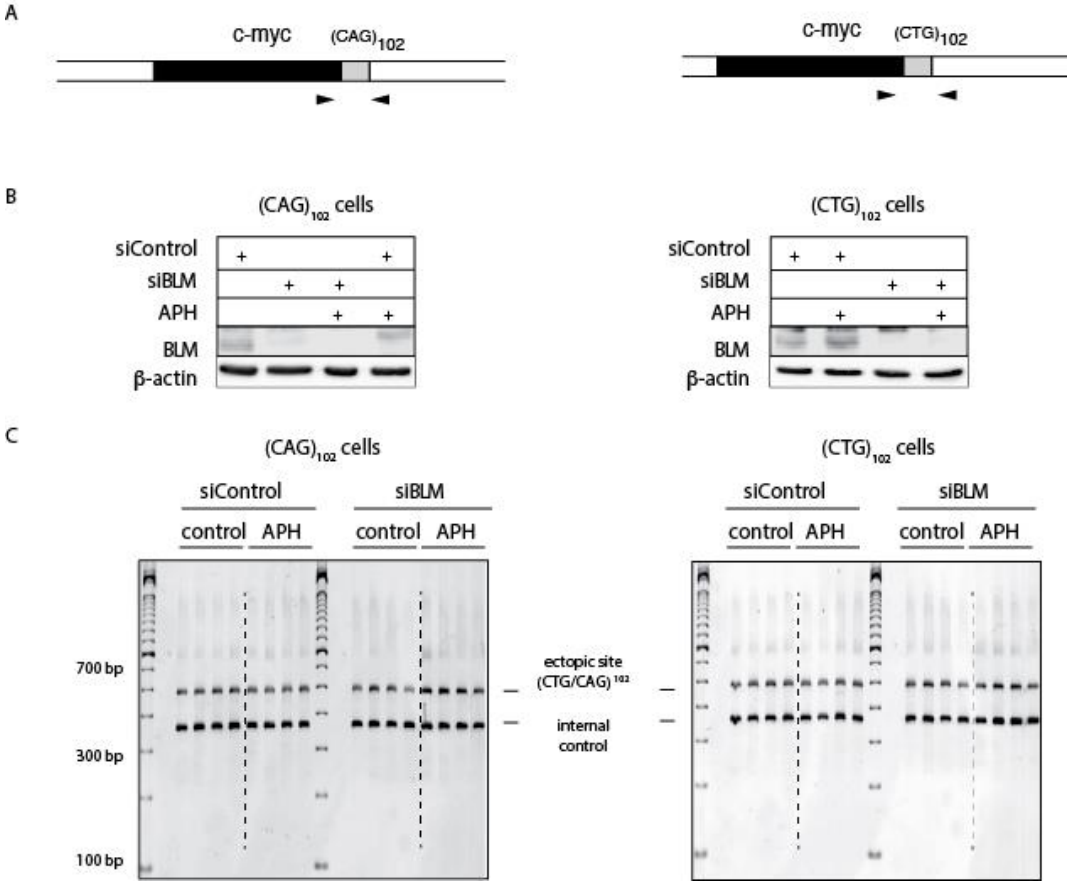

Figure 50. BLM knockdown does not lead to loss of ectopic (CTG)·(CAG) microsatellite signal in $(CTG)_{102}$·$(CAG)_{102}$ cells treated with aphidicolin. **(A)** Diagram of the integration site in $(CAG)_{102}$·$(CTG)_{102}$ cell lines. **(B)** Whole cell extracts were isolated after treatment of cells with siRNA against BLM and aphidicolin for a total of five transfections, or parallel untreated cultures, and immunoblotted for BLM. **(C)** Small pool PCR results with primers flanking the ectopic $(CAG)_{102}$·$(CTG)_{102}$ repeats and primers for a site without microsatellites serving as an internal control.

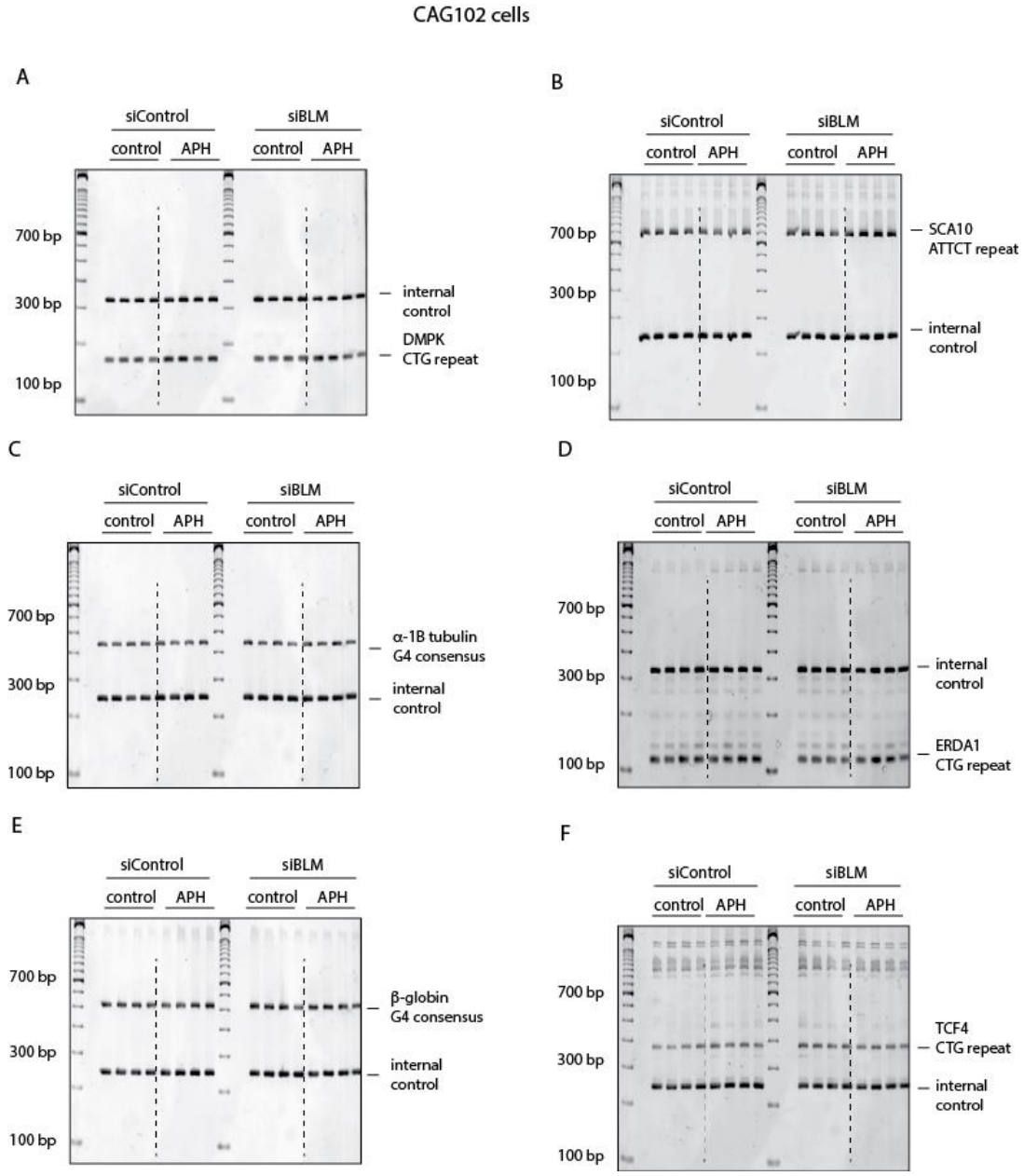

Figure 51. BLM knockdown does not lead to loss of PCR signal at multiple endogenous sites with aphidicolin treatment. Small pool PCR results of CAG_{102} cells treated with siBLM and aphidicolin showing no patterns of instability at multiple endogenous microsatellites.

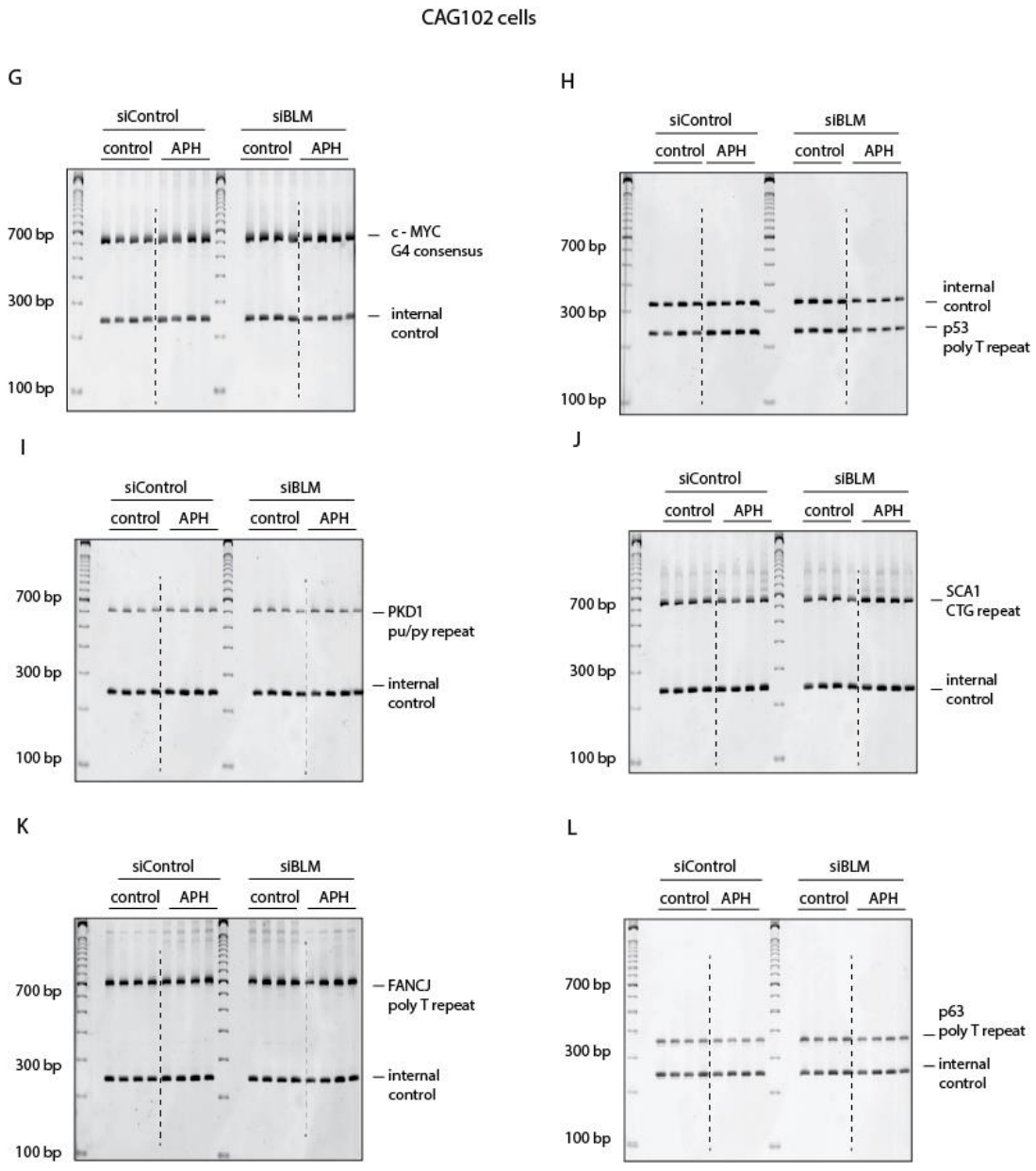

Figure 51 (continued). BLM knockdown does not lead to loss of PCR signal at multiple endogenous sites with aphidicolin treatment. Small pool PCR results of CAG_{102} cells treated with siBLM and aphidicolin showing no patterns of instability at multiple endogenous microsatellites.

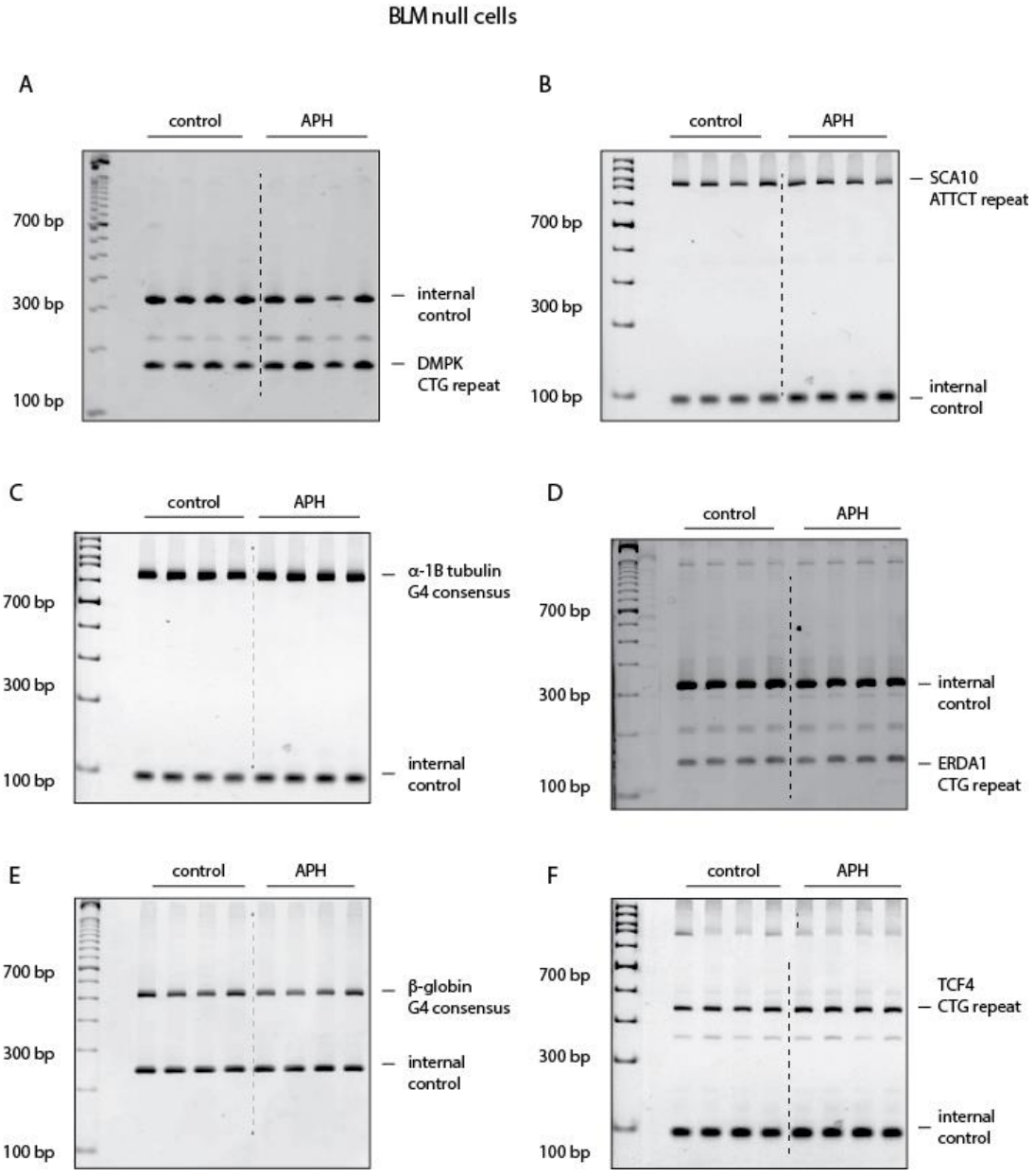

Figure 52. BLM null cells exposed to aphidicolin. Small pool PCR results for DNA from BLM null patient cells treated with aphidicolin.

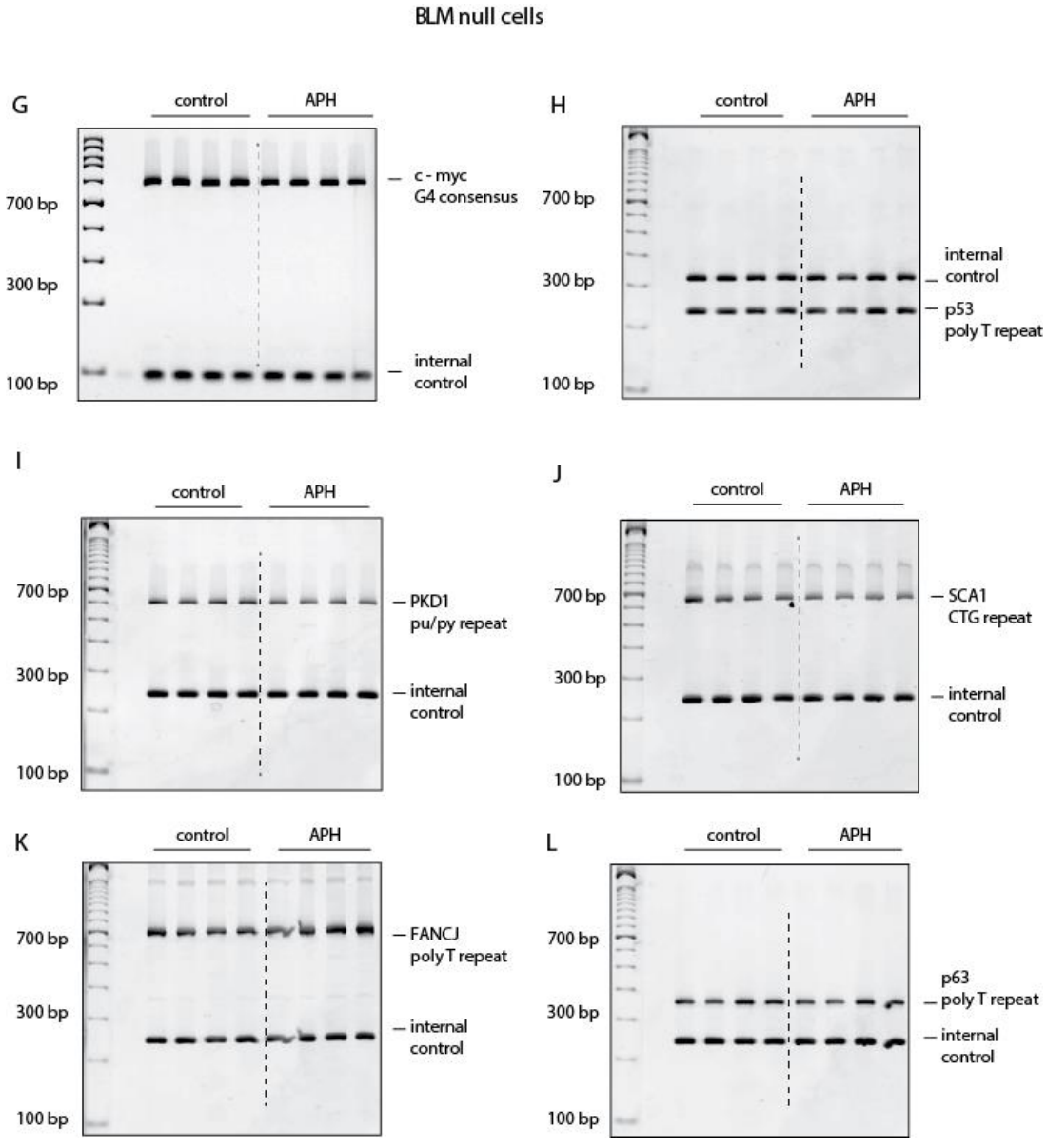

Figure 52 (continued). BLM null cells exposed to aphidicolin. Small pool PCR results for DNA from BLM null patient cells treated with aphidicolin.

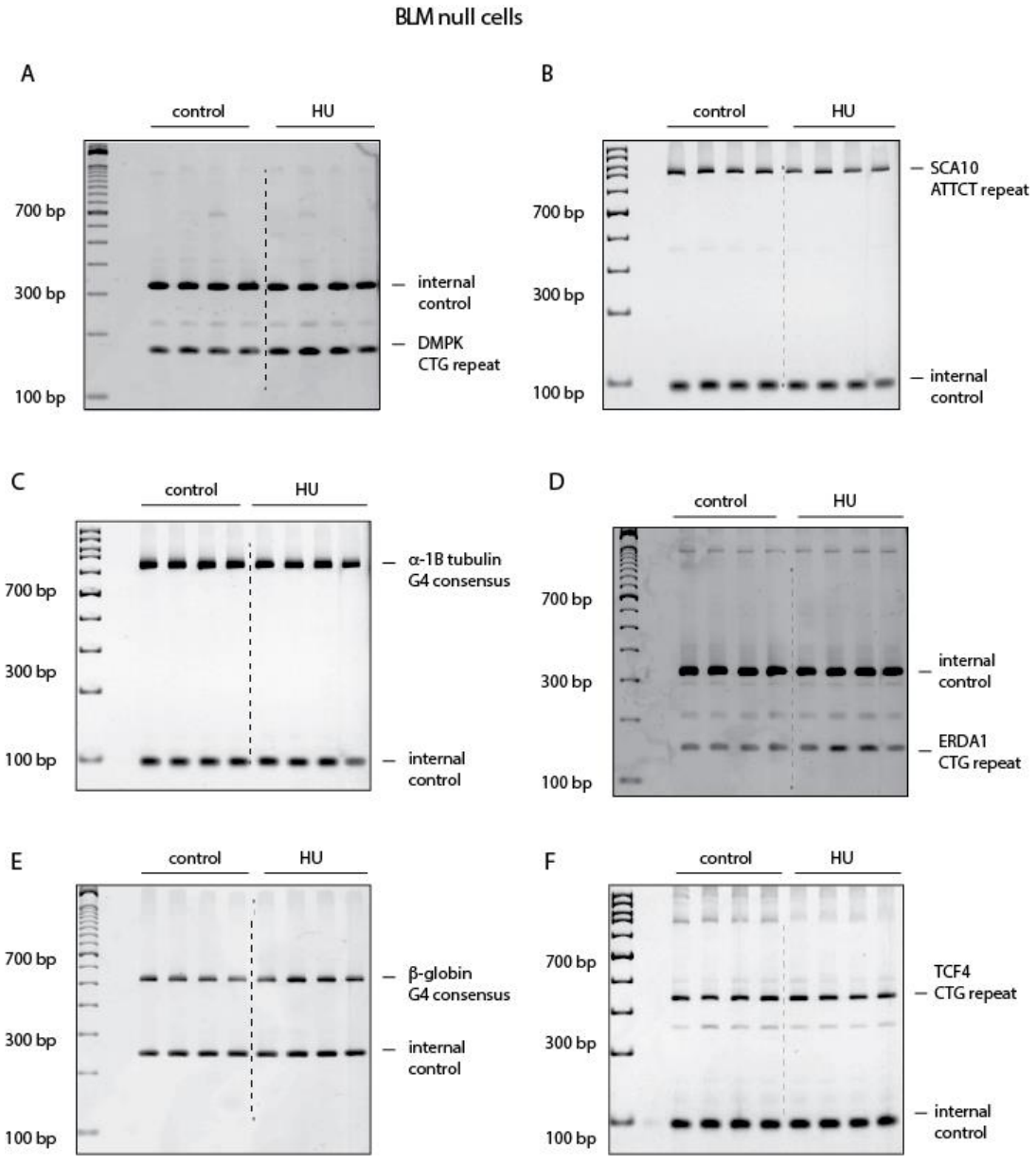

Figure 53. BLM null cells exposed to hydroxyurea. Small pool PCR results for DNA from BLM null patient cells treated with hydroxyurea.

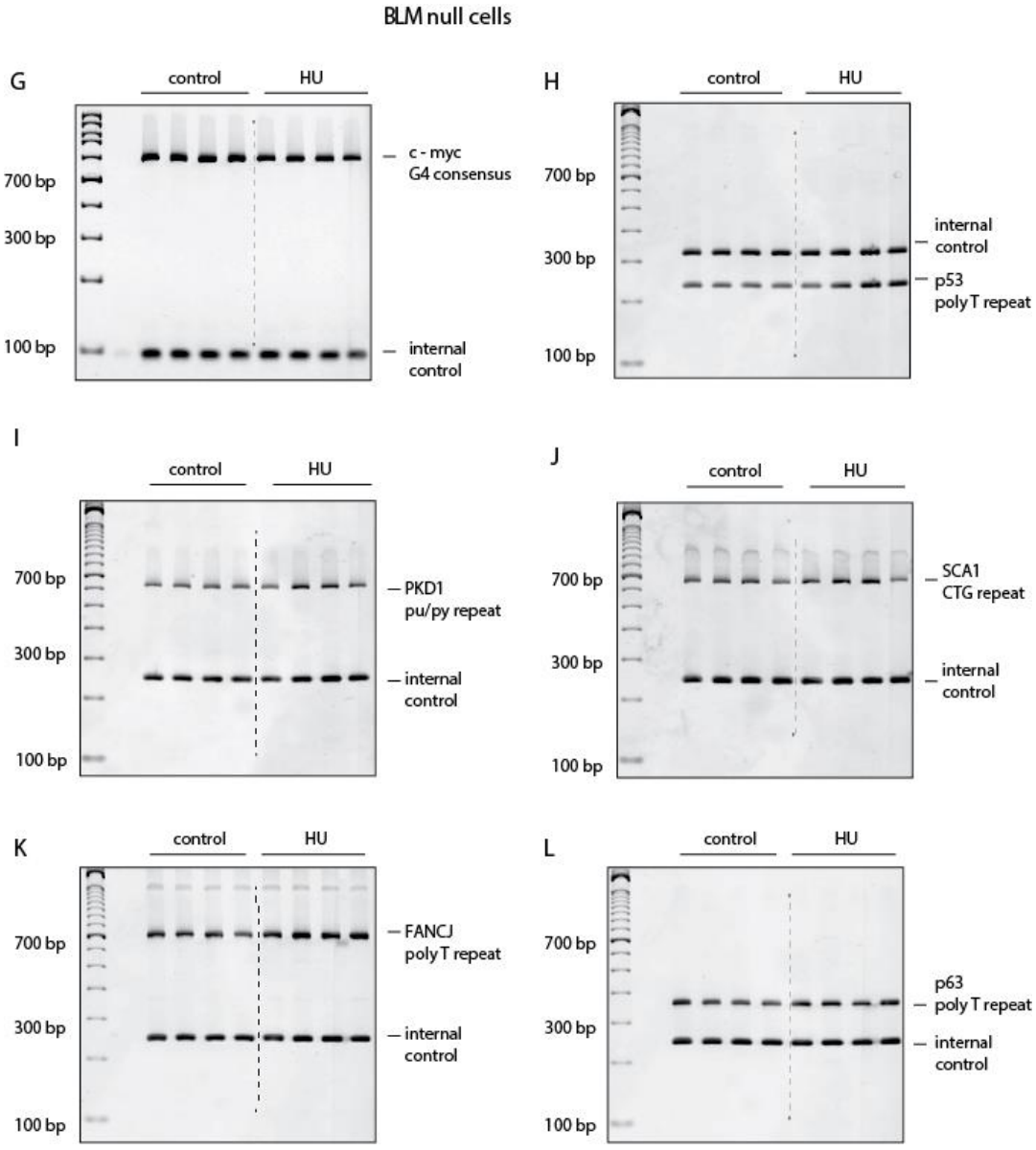

Figure 53 (continued). BLM null cells exposed to hydroxyurea. Small pool PCR results for DNA from BLM null patient cells treated with hydroxyurea.

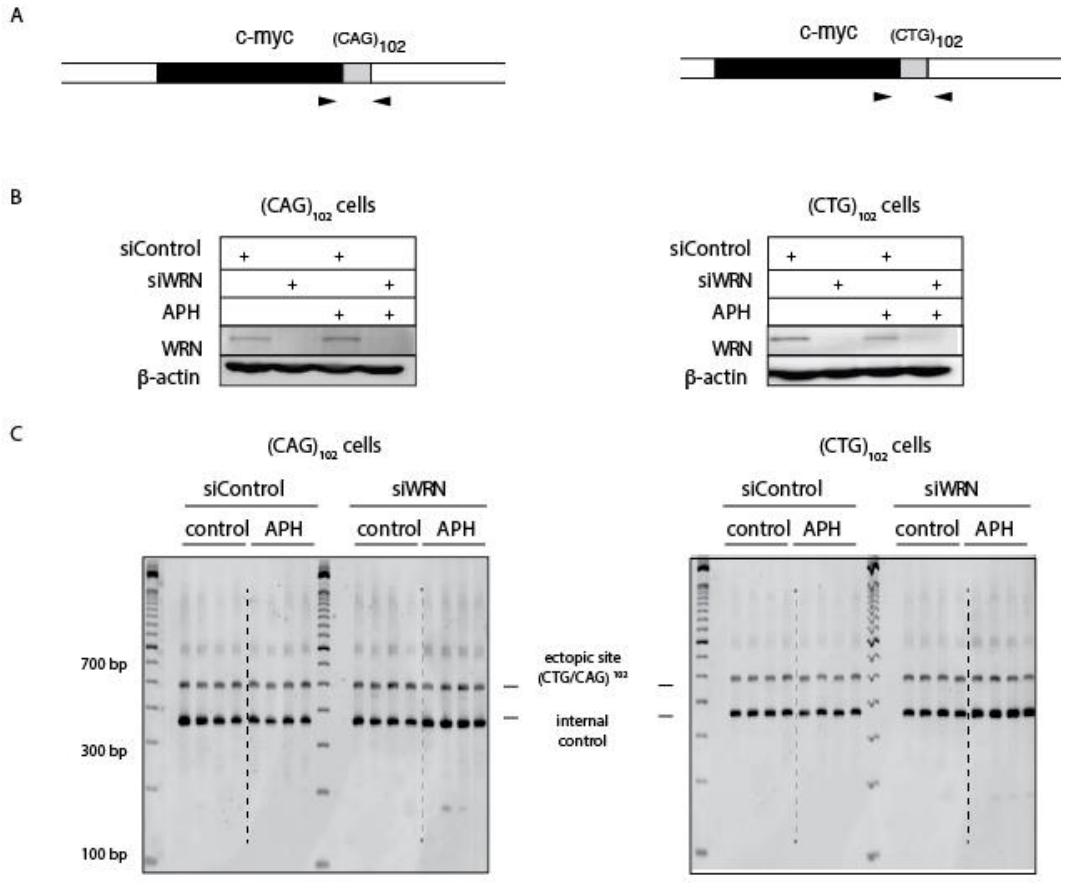

Figure 54. WRN knockdown does not lead to loss of ectopic (CTG)·(CAG) microsatellite signal in $(CTG)_{102}$·$(CAG)_{102}$ cells treated with aphidicolin. **(A)** Diagram of the integration site in $(CAG)_{102}$·$(CTG)_{102}$ cell lines. **(B)** Whole cell extracts were isolated after treatment of cells with siRNA against WRN helicase and aphidicolin for a total of five transfections, or parallel untreated cultures, and immunoblotted for BLM. **(C)** Small pool PCR results with primers flanking the ectopic $(CAG)_{102}$·$(CTG)_{102}$ repeats and primers for a site without microsatellites. serving as an internal control.

microsatellite instability (loss of spPCR signal) at the ectopic site (CTG)·(CAG) repeats (Figure 50C) and endogenous microsatellites (Figure 51). spPCR results were confirmed in primary Bloom's Syndrome patient fibroblast cells (BLM null) treated with aphidicolin (Figure 52) or hydroxyurea (Figure 53) suggesting BLM helicase activity is dispensable for FANCJ to stabilize microsatellite sequences.

FANCJ can function independently of WRN helicase activity

Similarly, we examined the requirement of WRN activity for microsatellite stabilization by FANCJ. CTG_{102}·CAG_{102} cells were depleted of WRN helicase with a pool of siRNAs (Figure 54B) and treated with aphidicolin. DNA isolated from cells treated with both WRN siRNA and aphidicolin showed a similar pattern of spPCR products compared to control counterparts at the ectopic site (CTG)·(CAG) repeats (Figure 54C) and endogenous microsatellites (Figure 55), in contrast to spPCR products produced from CTG_{102}·CAG_{102} cells treated with FANCJ siRNA and aphidicolin (Figure 7). Lack of spPCR signal loss at endogenous microsatellite sequences was confirmed in primary Werner's Syndrome patient cells exposed to aphidicolin (Figure 56) and hydroxyurea (Figure 57) to induce replication stress signifying WRN helicase function is expendable for FANCJ to maintain microsatellite stability.

A normal primary fibroblast cell line was treated with aphidicolin (Figure 58) or hydroxyurea (Figure 59) as a control for primary BLM helicase null and primary WRN helicase null cell lines exposed to replicative stress. Under both conditions, no loss of PCR signal at endogenous microsatellite sequences was observed.

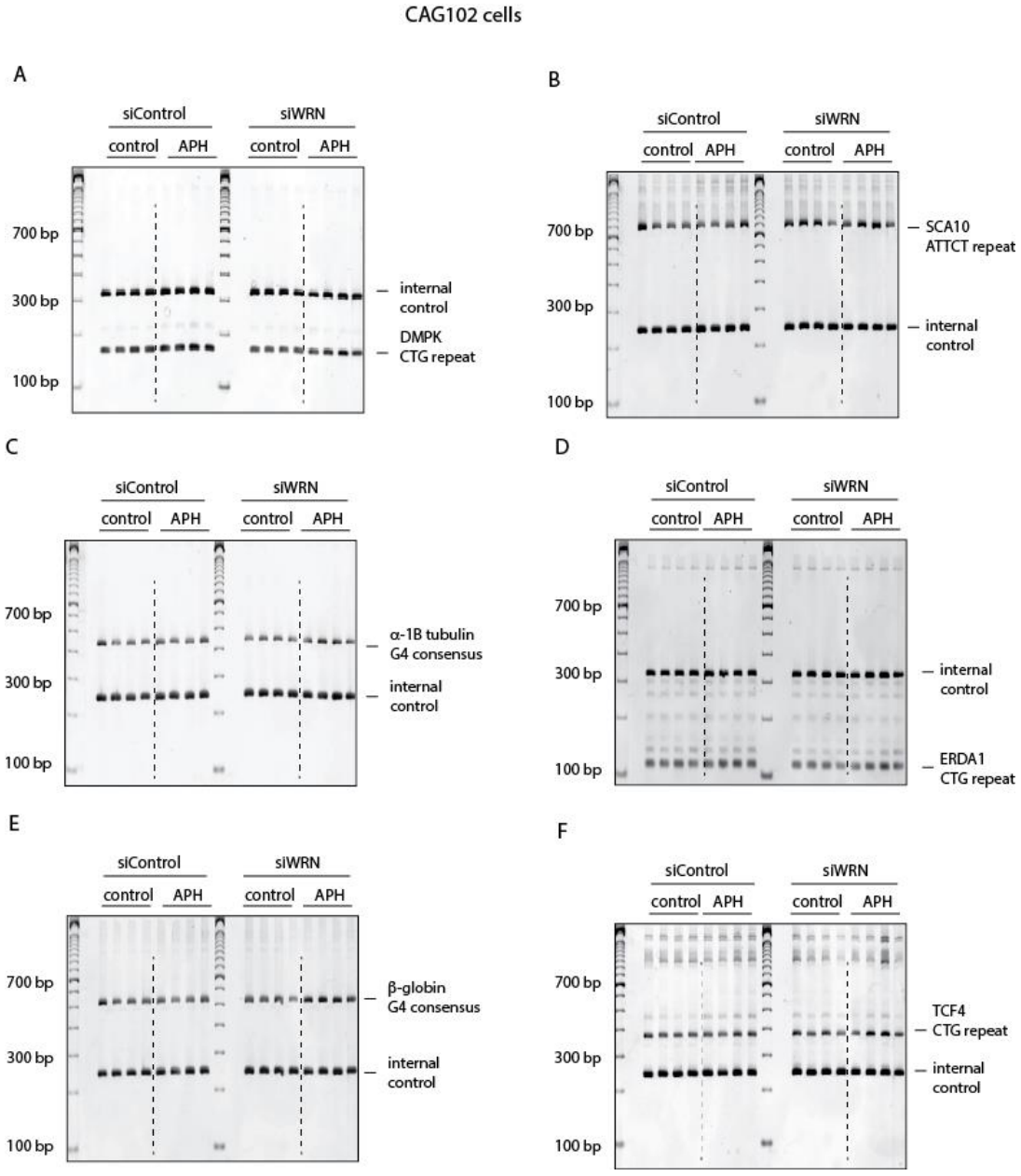

Figure 55. WRN knockdown does not lead to loss of PCR signal at multiple endogenous sites with aphidicolin treatment. Small pool PCR results of CAG_{102} cells treated with siWRN and aphidicolin showing no patterns of instability at multiple endogenous microsatellites.

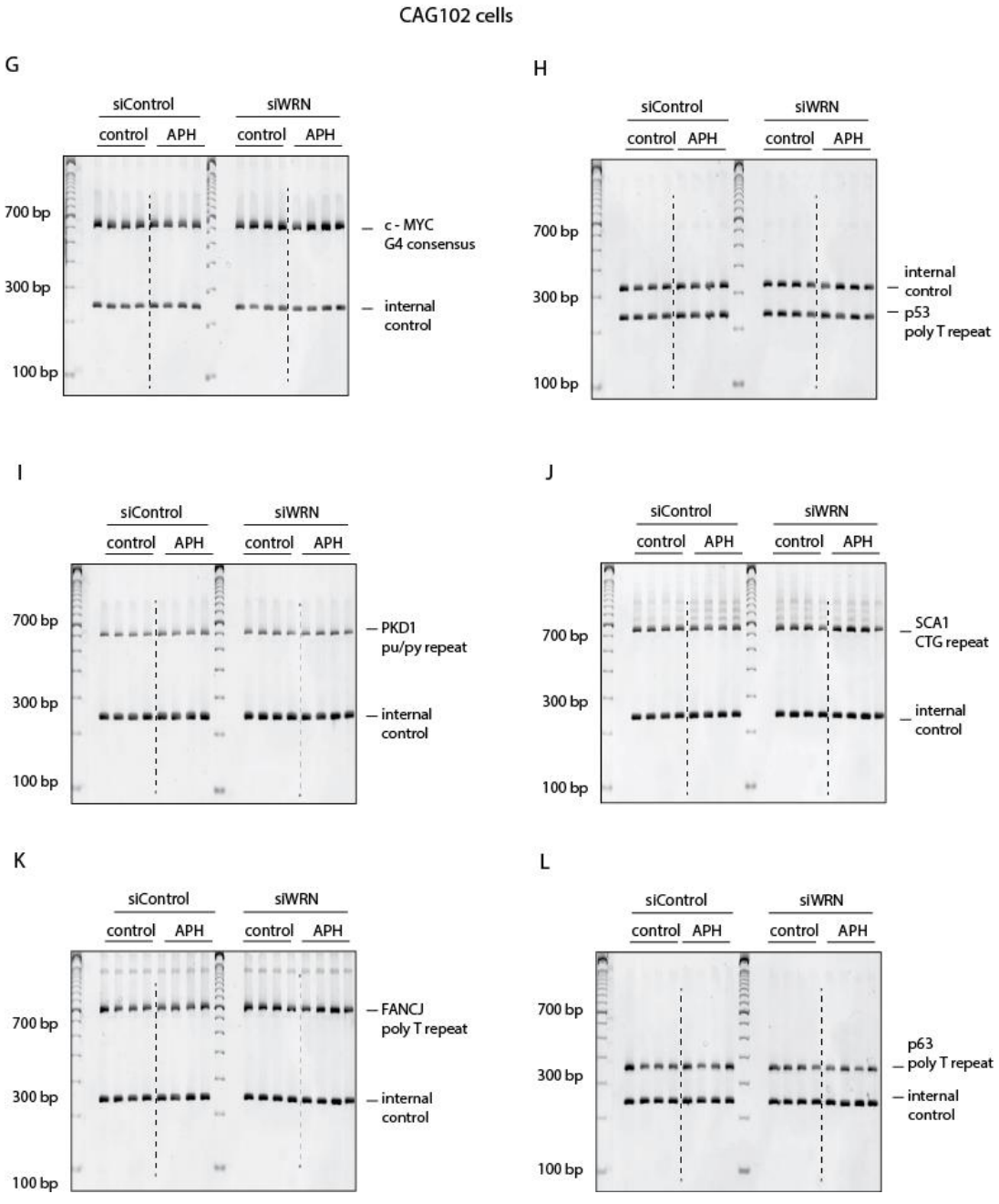

Figure 55 (continued). WRN knockdown does not lead to loss of PCR signal at multiple endogenous sites with aphidicolin treatment. Small pool PCR results of CAG_{102} cells treated with siWRN and aphidicolin showing no patterns of instability at multiple endogenous microsatellites.

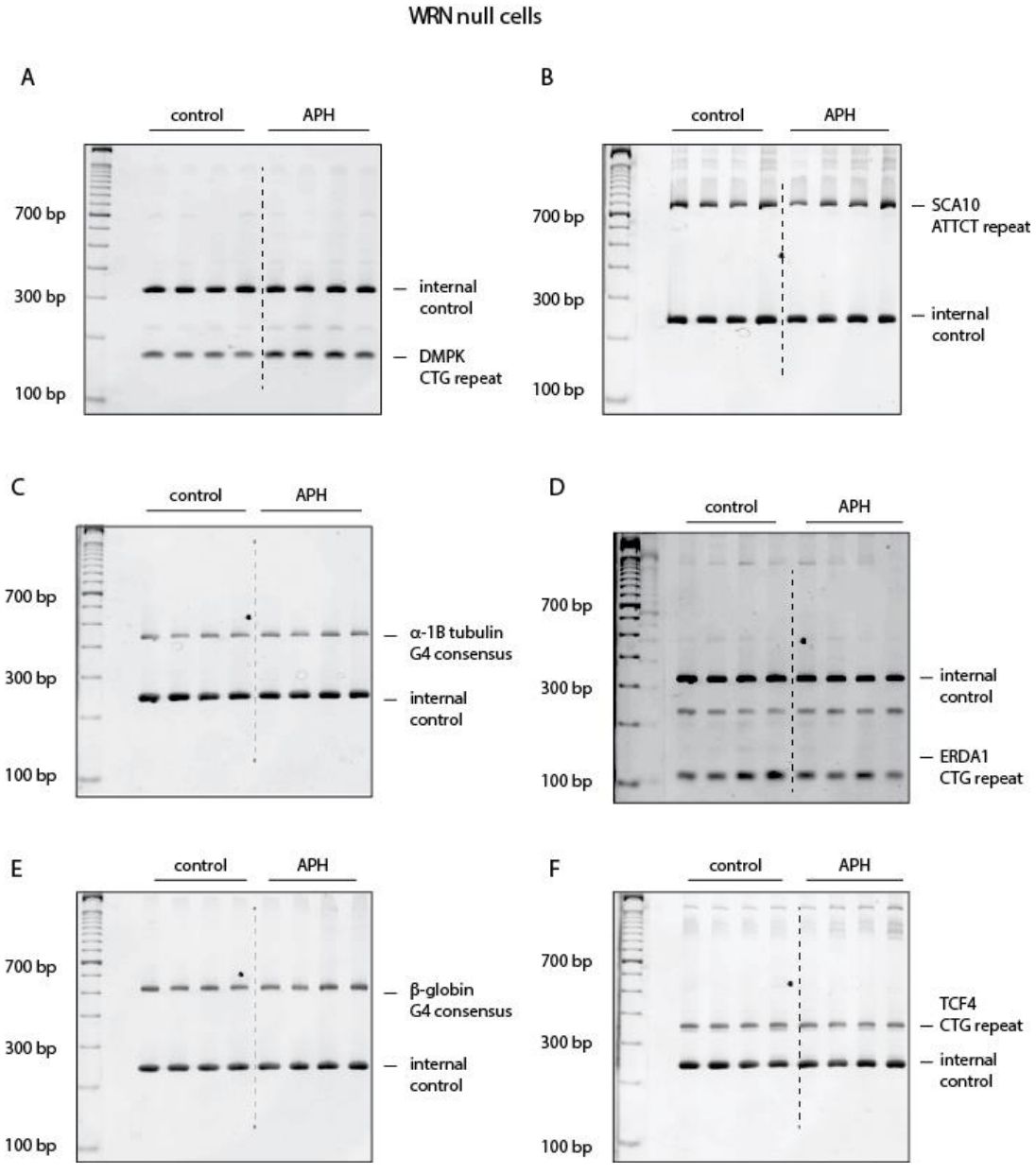

Figure 56. Aphidicolin treatment of WRN null patient cells. Small pool PCR across repeated sequences in DNA from WRN null patient cells treated with aphidicolin.

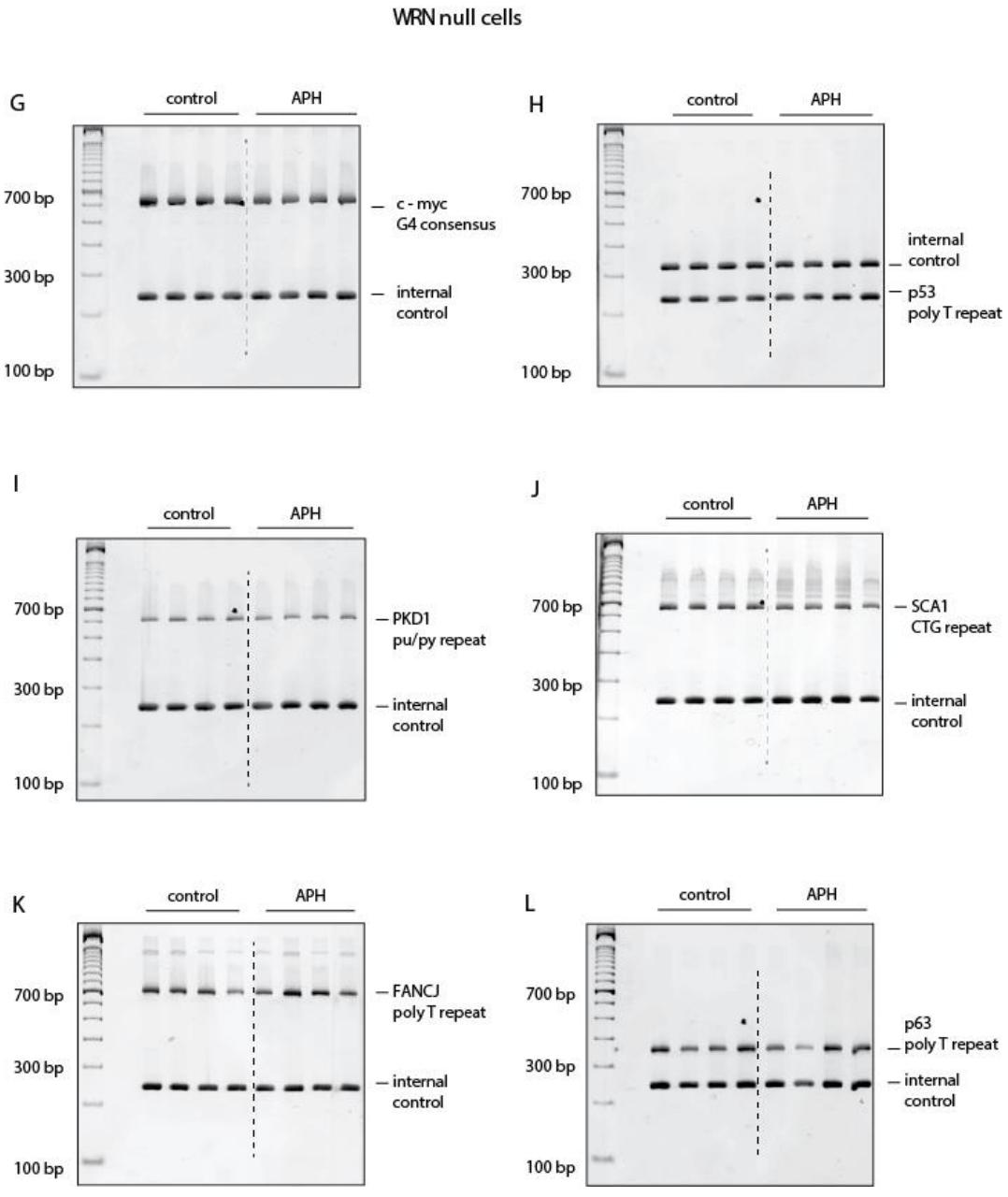

Figure 56 (continued). Aphidicolin treatment of WRN null patient cells. Small pool PCR across repeated sequences in DNA from WRN null patient cells treated with aphidicolin.

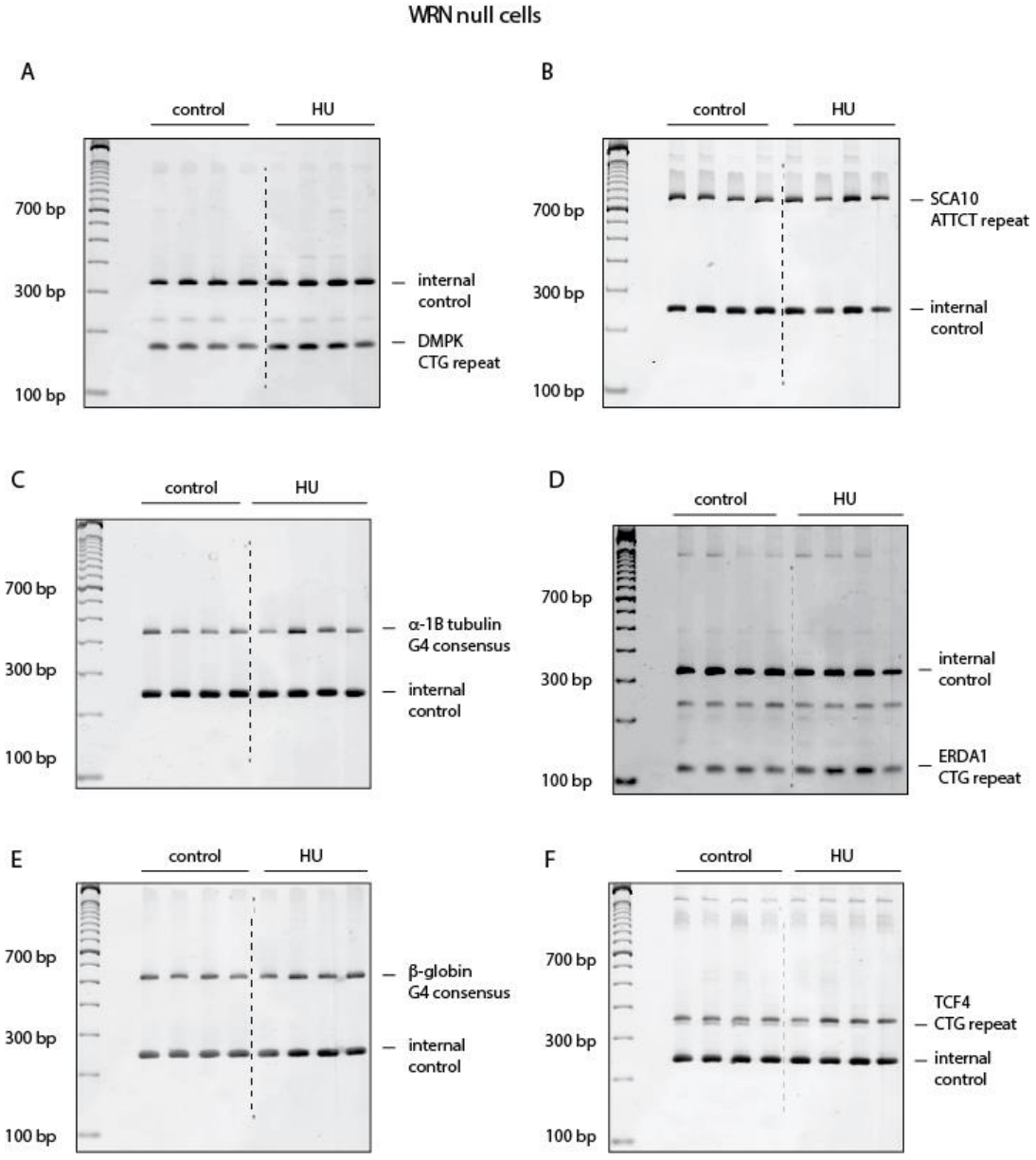

Figure 57. Hydroxyurea treatment of WRN null patient cells. Small pool PCR across repeated sequences in DNA from WRN null patient cells treated with hydroxyurea.

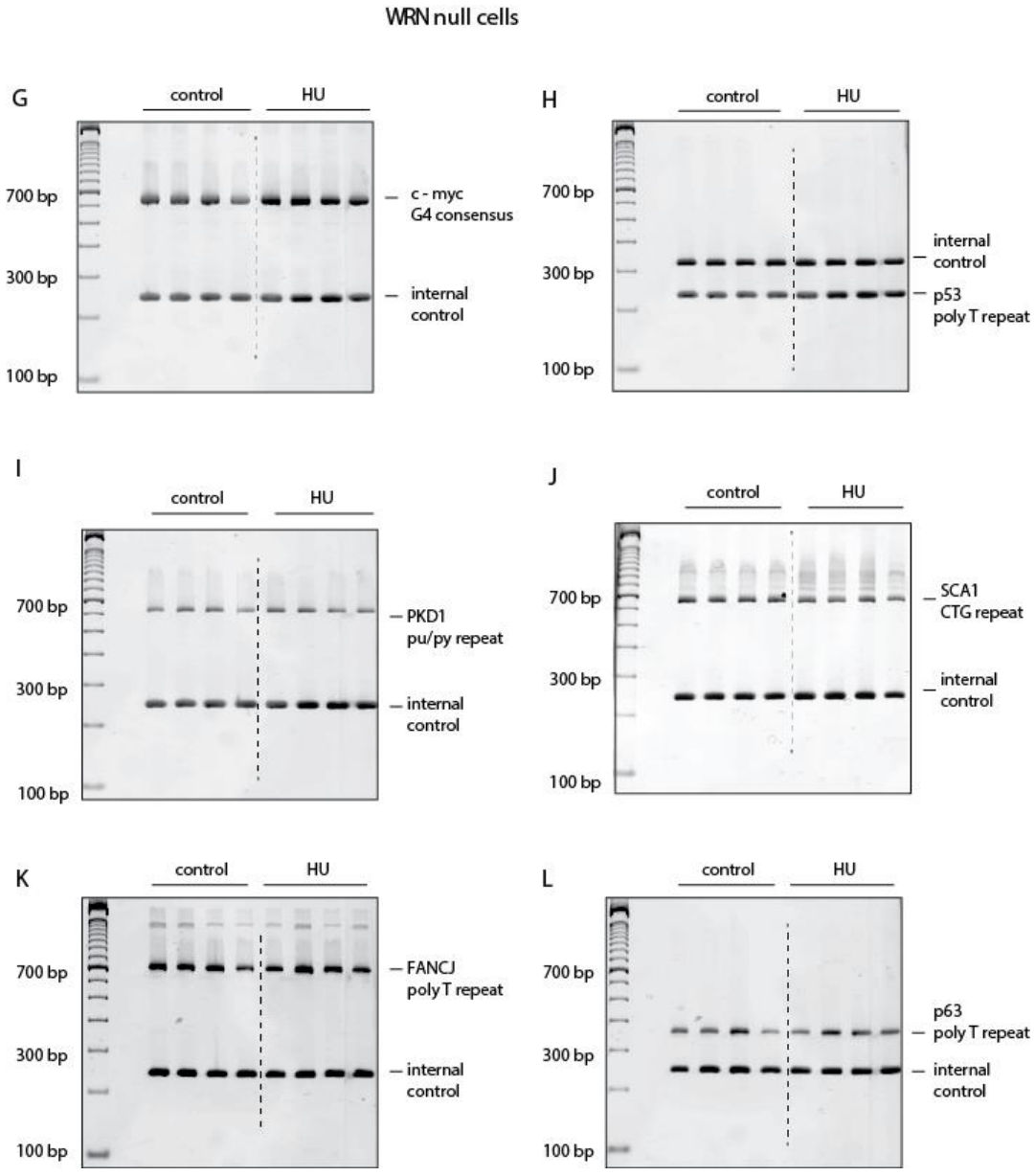

Figure 57 (continued). Hydroxyurea treatment of WRN null patient cells. Small pool PCR across repeated sequences in DNA from WRN null patient cells treated with hydroxyurea.

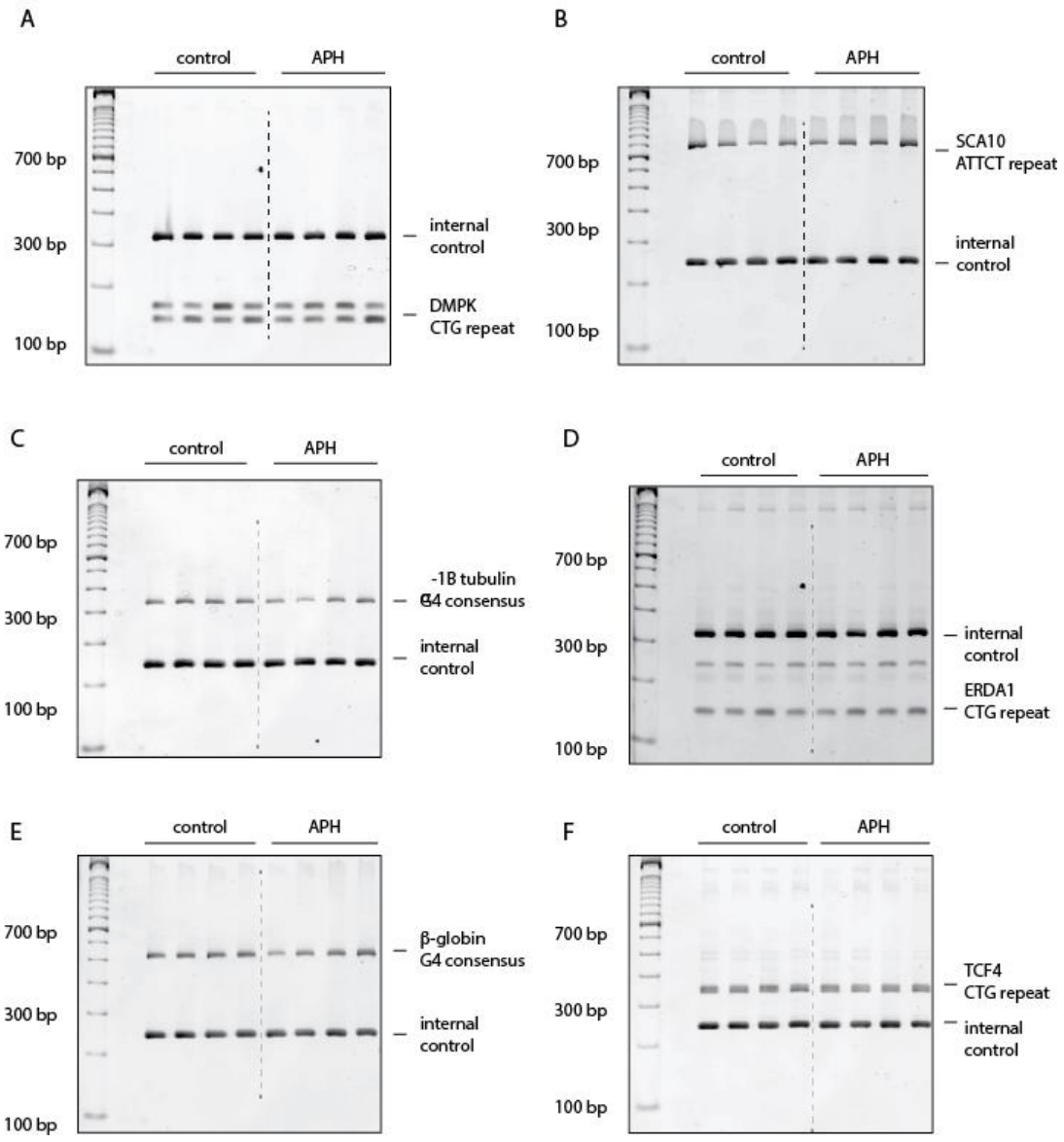

Figure 58. Primary normal fibroblast cells treated with aphidicolin. Small pool PCR across repeated sequences in DNA from primary normal fibroblasts treated with aphidicolin.

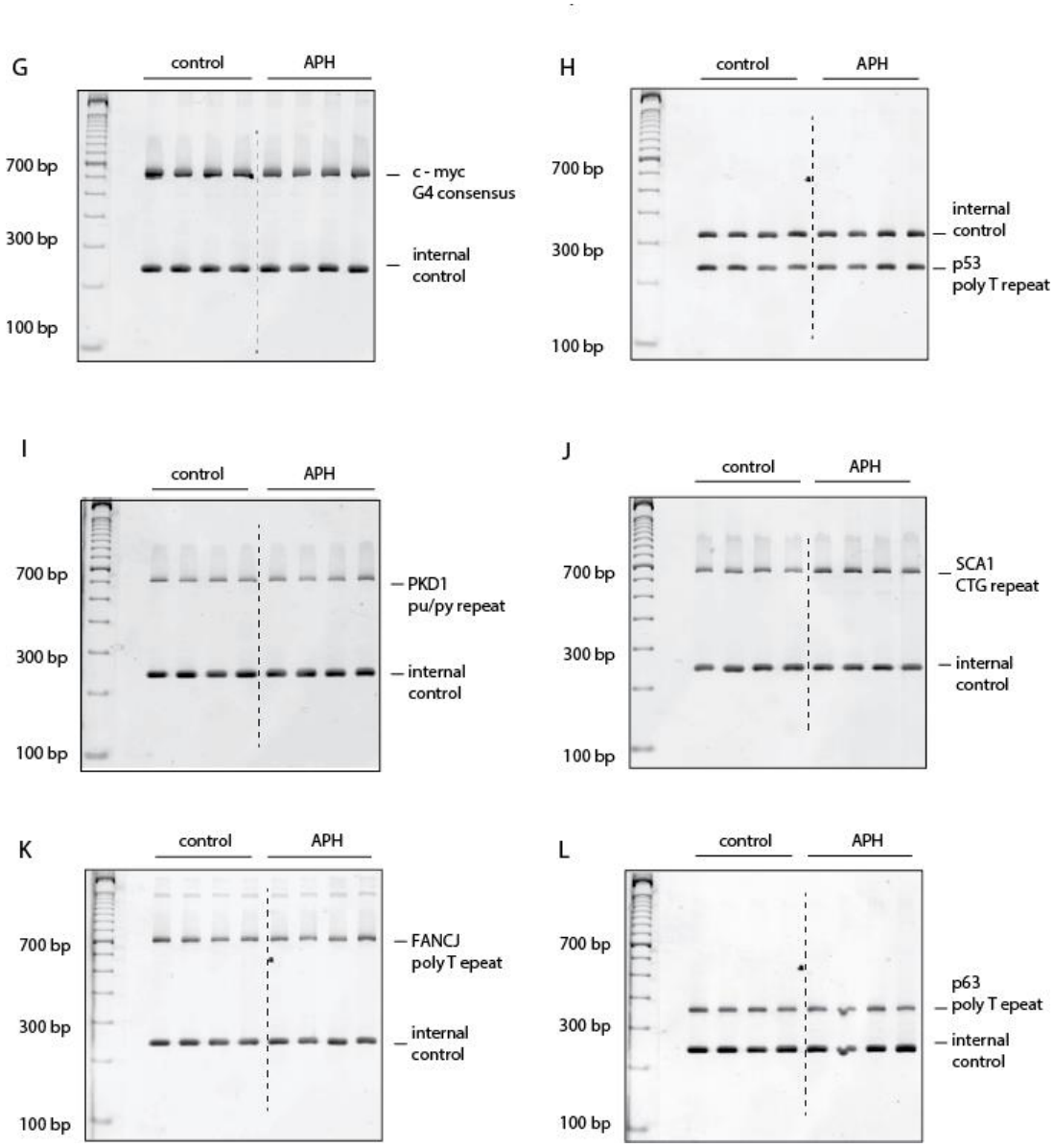

Figure 58 (continued). Primary normal fibroblast cells treated with aphidicolin. Small pool PCR across repeated sequences in DNA from primary normal fibroblasts treated with aphidicolin.

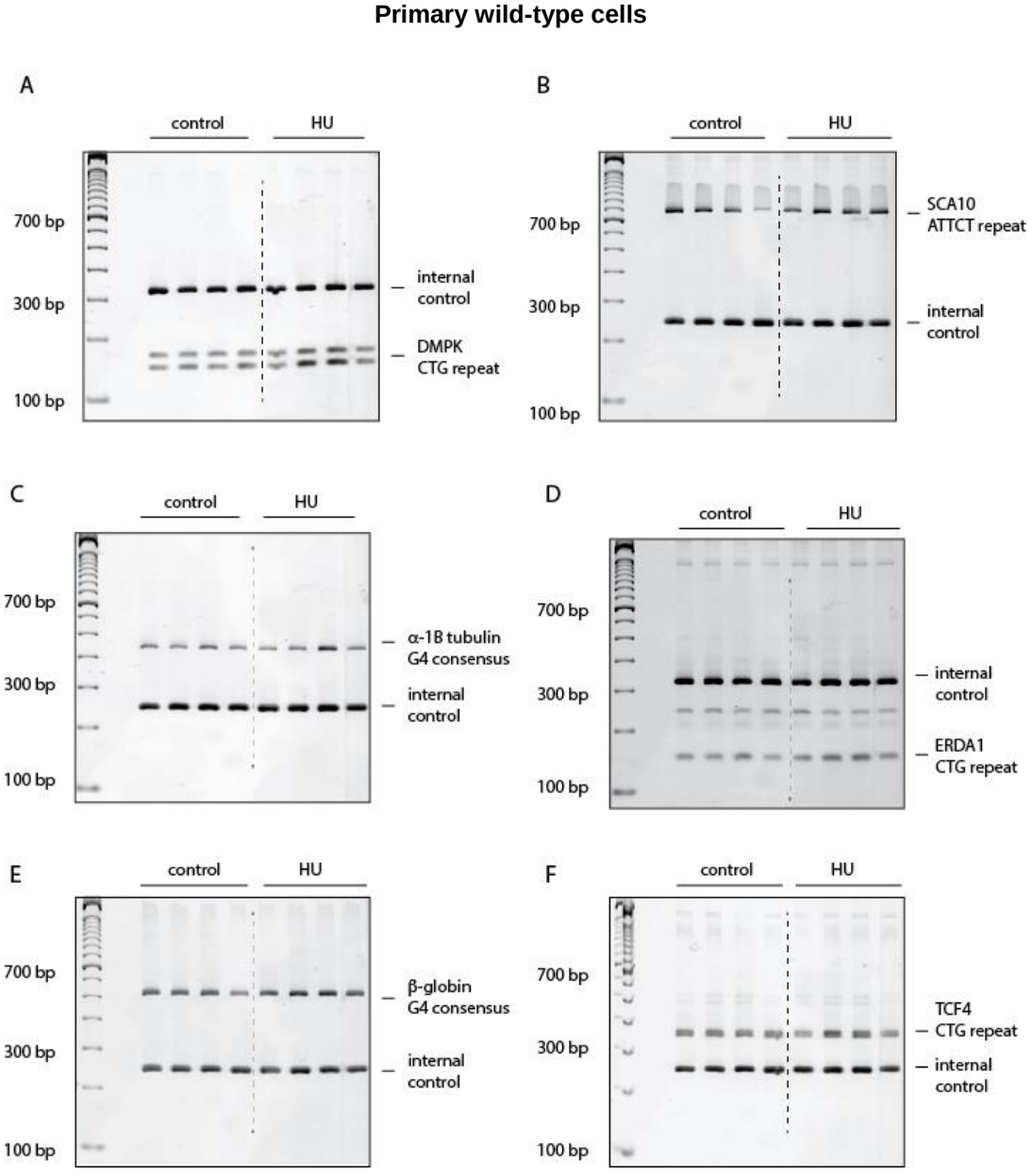

Figure 59. Primary normal fibroblast cells treated with hydroxyurea. Small pool PCR across repeated sequences in DNA from primary normal fibroblasts treated with hydroxyurea.

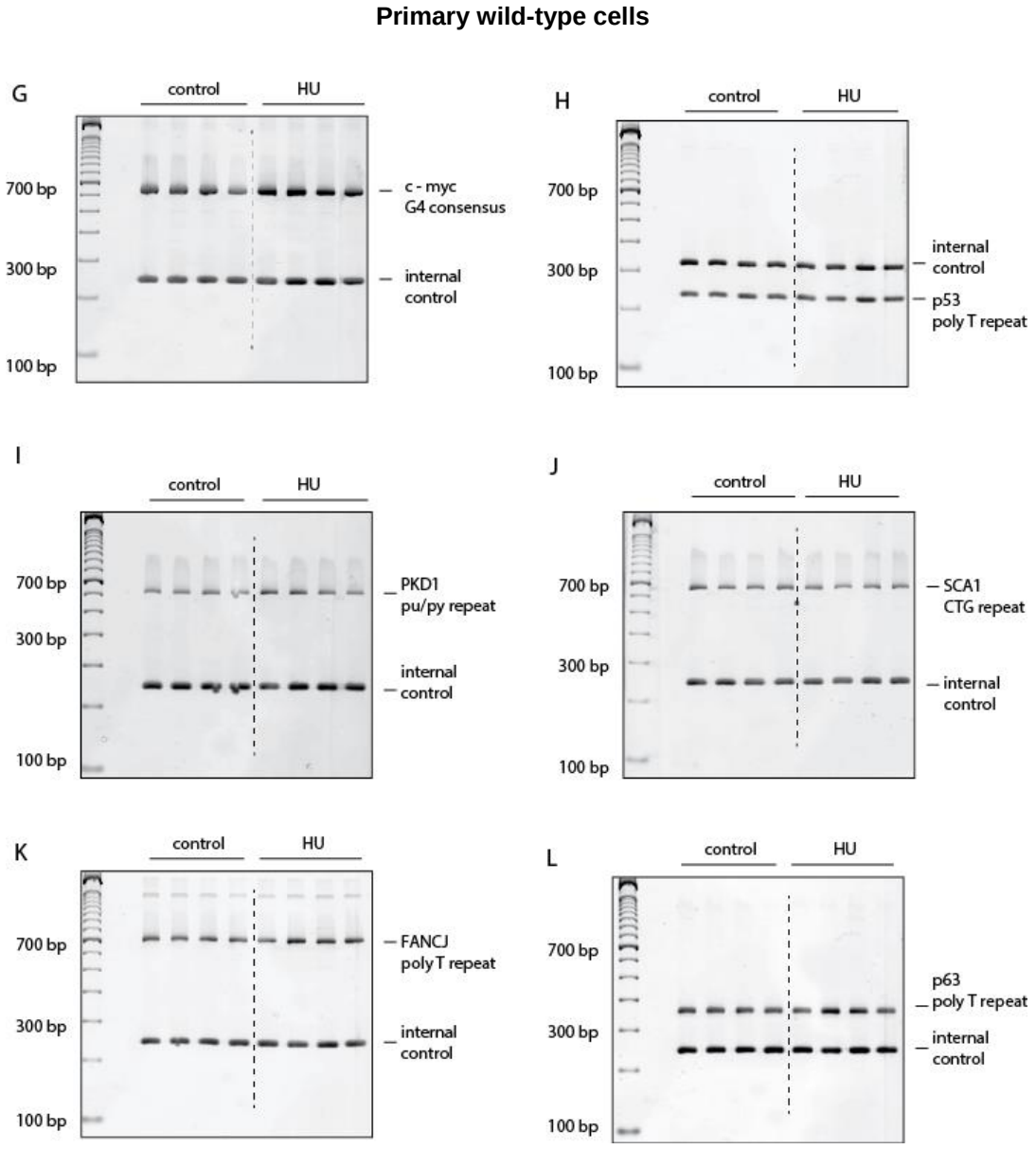

Figure 59 (continued). Primary normal fibroblast cells treated with hydroxyurea. Small pool PCR across repeated sequences in DNA from primary normal fibroblasts treated with hydroxyurea.

Summary

The present data focus on understanding the potential roles for BLM helicase or WRN helicase in FANCJ microsatellite stabilization. Recent studies have indicated a cooperative effort between FANCJ and BLM/WRN helicase to maintain genome integrity. First, we examined the effect of loss of BLM helicase on microsatellite stability. Replicative stress does not lead to loss of spPCR signal at the ectopic site (CTG)·(CAG) repeats and endogenous microsatellites in BLM helicase depleted cells suggesting BLM helicase activity is nonessential for FANCJ to stabilize microsatellites. Similarly, induction of replicative stress in WRN depleted and null cells did result in loss of spPCR signal indicating WRN helicase is dispensable for FANCJ mediated microsatellite stabilization. Taken together, these results suggest FANCJ can function in the absence of BLM helicase or WRN helicase to maintain microsatellite stability.

V. FANCJ mutagenesis

FANCJ stabilizes microsatellites independent of BRCA1, TopBP1 and BLM interaction

Work presented above demonstrates a role for FANCJ in the maintenance of genome stability (Figure 7). However, the domain(s) necessary for this function of FANCJ remain(s) unknown. Towards characterizing this role we obtained an immortalized Faconi anemia complementation group J patient cell line expressing a biallelic mutant of FANCJ ($FANCJ^{R798X}$). This mutation results in an arginine switch to a premature stop codon. The protein product of the mutagenized gene eliminates the

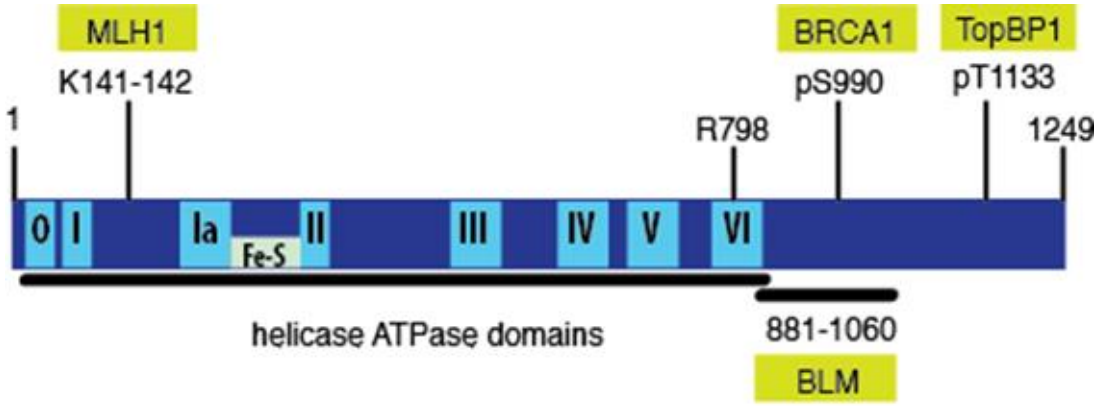

Figure 60: Schematic of FANCJ protein. FANCJ consists of a helicase (comprised of 7 helicase domains, BRCA1 binding domain, TopBP1 binding domain, RPA binding domain, BLM binding domain, MLH1 binding domain, iron-sulfur cluster (Fe-S) and nuclear localization signal. (Wu and Brosh, 2009)

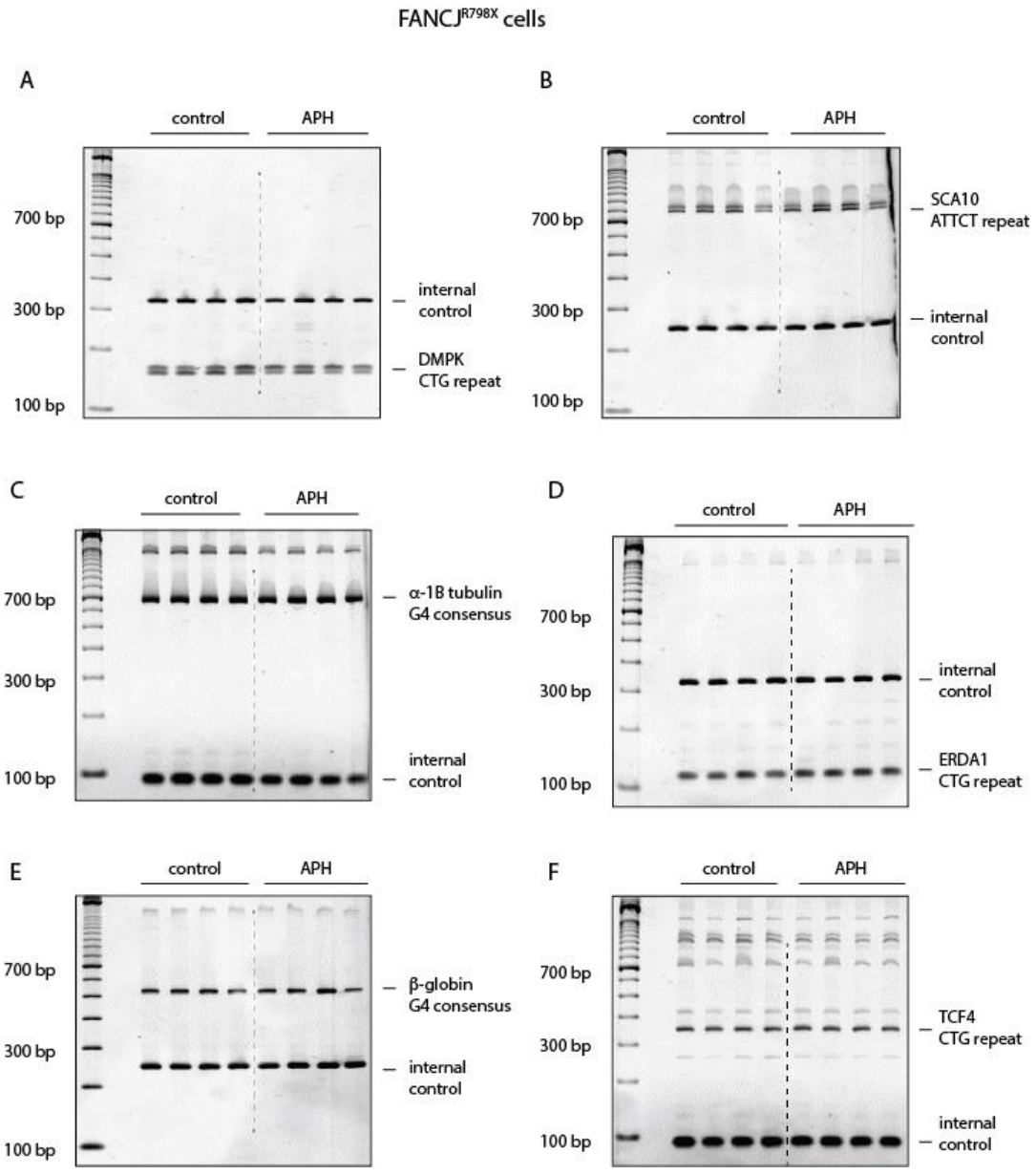

Figure 61. FANCJ^{R798X} mutant cells are insensitive to microsatellite loss with aphidicolin treatment. Small pool PCR across endogenous repeated sequences in DAN from biallelic FANCJ^{R798X} patient fibroblasts with aphidicolin.

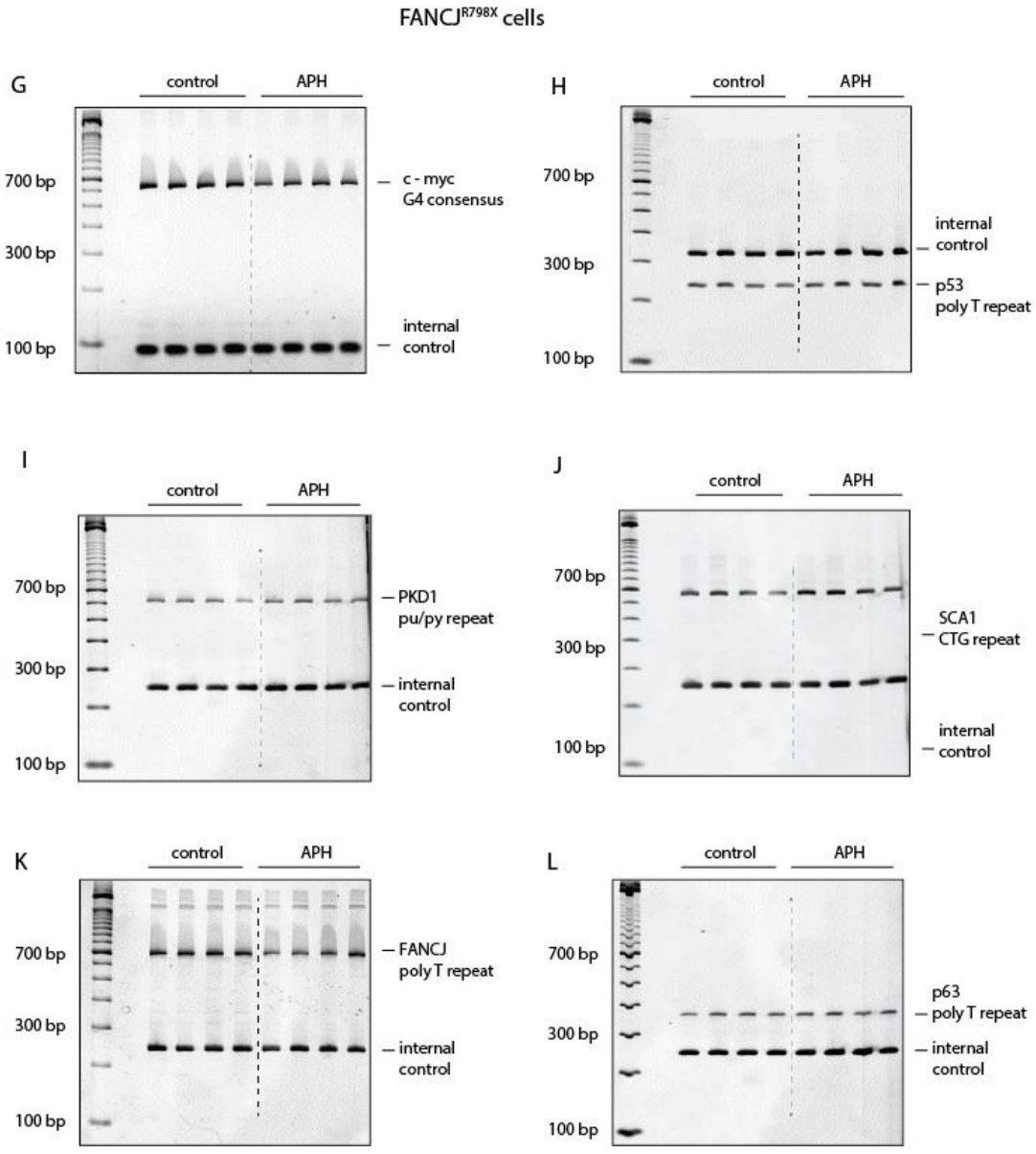

Figure 61 (continued). FANCJ^{R798X} mutant cells are insensitive to microsatellite loss with aphidicolin treatment. Small pool PCR across endogenous repeated sequences in DAN from biallelic FANCJ^{R798X} patient fibroblasts with aphidicolin.

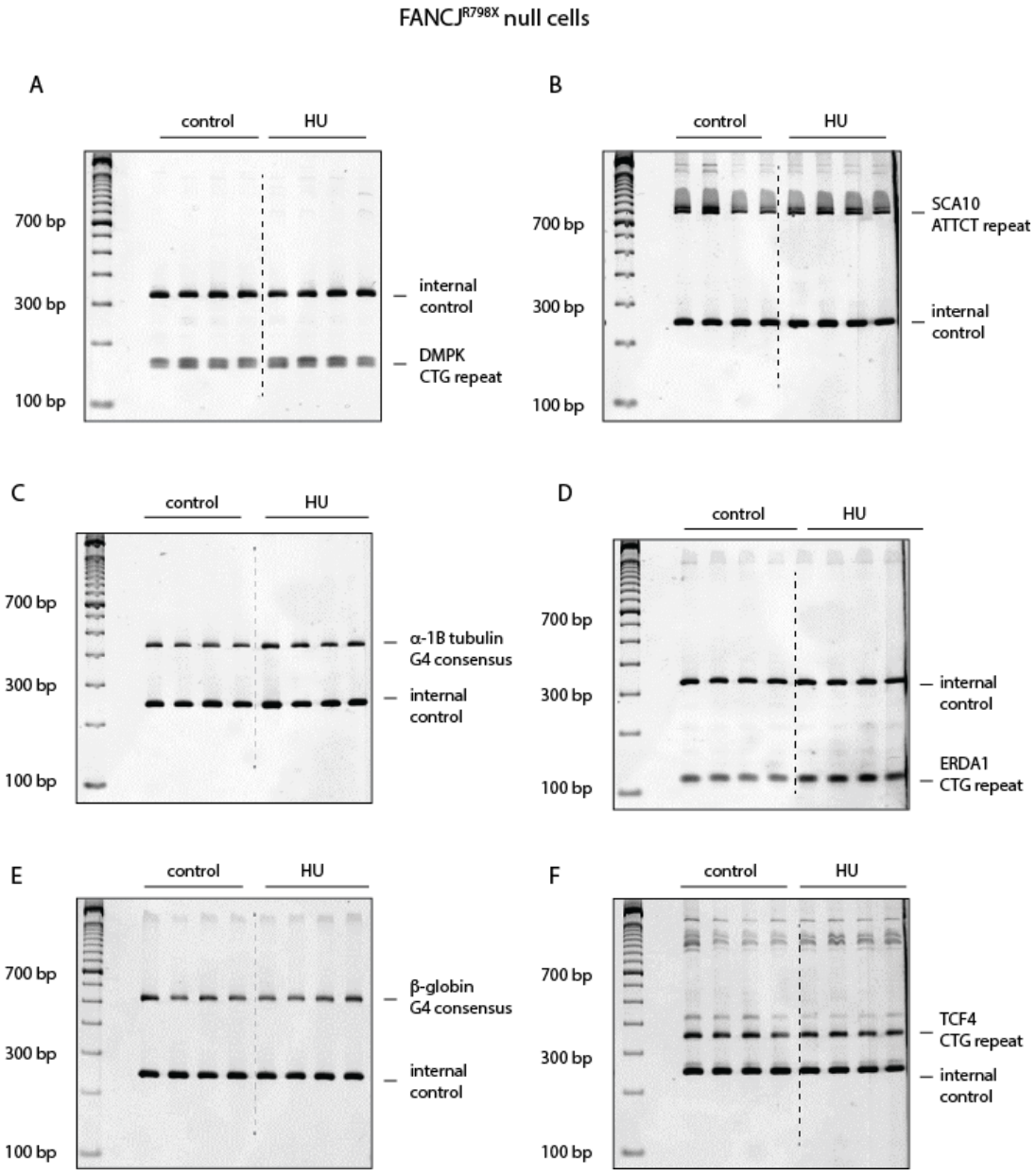

Figure 62. FANCJ^{R798X} mutant cells are insensitive to microsatellite loss with hydroxyurea treatment. Small pool PCR across endogenous repeated sequences in DAN from biallelic FANCJ^{R798X} patient fibroblasts with hydroxyurea.

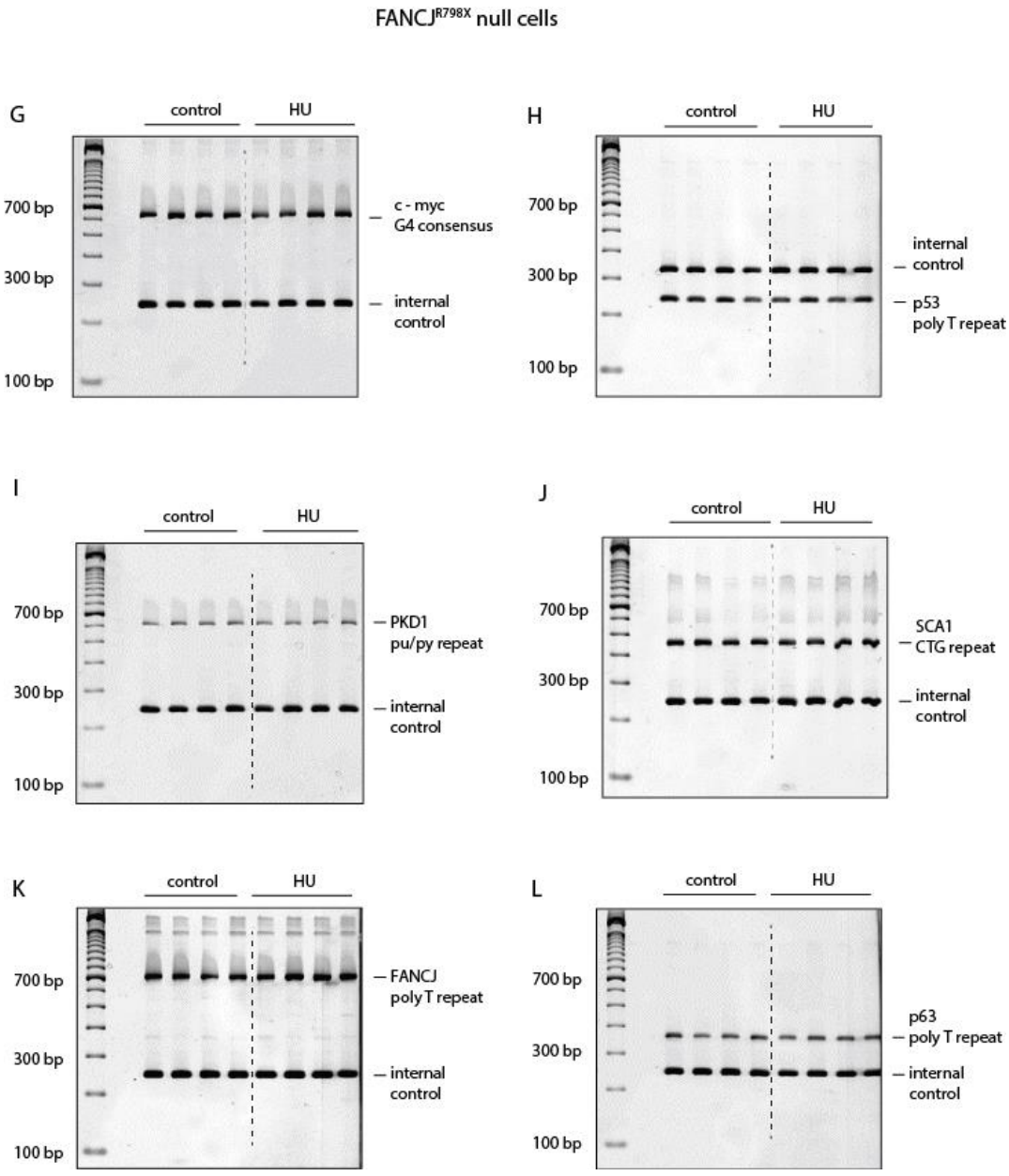

Figure 62 (continued). FANCJ^{R798X} mutant cells are insensitive to microsatellite loss with hydroxyurea treatment. Small pool PCR across endogenous repeated sequences in DAN from biallelic FANCJ^{R798X} patient fibroblasts with hydroxyurea.

following previously identified functional domains: BRCA1 binding domain, TopBP1 binding domain, and BLM helicase binding domain (Figure 60). Recent research suggests each domain is necessary to allow FANCJ to function properly to unwind noncanonical DNA structures and promote genome stability in the presence of interstrand crosslinking agents (Cantor et al., 2001; Gong et al., 2010; Greenberg et al., 2006; Leung et al., 2011; Suhasini and Brosh, 2012; Suhasini et al., 2011). Therefore, it is feasible that these domains are essential for FANCJ to function as a protector of microsatellite stability. In addition, *in vitro* analysis of FANCJ^{R798X} recombinant protein isolated from Sf9 insect cells shows a loss of helicase activity with this mutation (London et al., 2008). To determine if these domains of FANCJ are essential for microsatellite stabilization, we treated FANCJ^{R798X} cells with either aphidicolin (Figure 61) or hydroxyurea (Figure 62) to induce replication stress and examined microsatellite stability via spPCR. PCR products revealed no loss of signal at endogenous microsatellite sequences, in contrast to FANCJ null cells exposed to replicative stress. If these domains were necessary for FANCJ to maintain microsatellite stability, similar patterns of instability would be observed in the FANCJ^{R798X} (Figure 61 and 62) and FANCJ (Figure 16 and 17) null cells. Since no instability was observed in FANCJ^{R798X} cells treated with aphidicolin or hydroxyurea, we conclude that the BRCA1, TopBP1, and BLM helicase binding domains are not required for FANCJ to stabilize microsatellites across the genome during replicative stress.

Summary

The experiments performed above aim to determine which domains of FANCJ are required for FANCJ to stabilize microsatellite sequences under replicative stress

conditions. Immortalized Fanconi anemia patient cells harboring a truncation mutation of FANCJ ($FANCJ^{R798X}$) abolishing BRCA1, TopBP1, and BLM helicase interacting domains were exposed to replicative stress. spPCR results showed no patterns of microsatellite instability indicating these domains are nonessential for FANCJ to stabilize microsatellite sequences.

DISCUSSION

The data presented here suggest a novel role for FANCJ helicase in the protection of microsatellite stability. Although FANCJ is known to unwind DNA G-quadruplex and triplex structures, it was not clear whether this activity extended to other noncanoncial DNA structures or was important *in vivo*. Here, we show *in vivo* in human cells that FANCJ helicase is essential to stabilize microsatellites prone to adopt noncanoncial DNA structures during replication stress. This unique function of FANCJ occurs independently of the FA repair pathway, irrespective of BLM helicase and WRN helicase activity, and without the interaction sites for BRCA1 or TopBP1.

FANCJ helicase in (CTG)·(CAG) repeat stability

Expansions of the (CTG)·(CAG) repeats in the 3' untranslated region of the DMPK gene cause several of the phenotypes associated with DM1 on a cellular level. In cell models, expansion or contraction of the (CTG)·(CAG) trinucleotide repeats can be induced with cycles of replication stress followed by recovery (Liu et al., 2012). The data presented here shows (CTG)·(CAG) repeats are sites of chromosome fragility with the depletion of FANCJ helicase in the presence of continuous replication stress, as might be experienced *in vivo*. Hence, the knockdown of FANCJ helicase in $(CTG)_{102}$·$(CAG)_{102}$ DM1 model cells treated with replicative stressors produced instability of the ectopic site (CTG)·(CAG) repeats. These results open the possibility that replication restart during repeated cycles of replicative stress and recovery induces a different pattern of instability

than continuous replication stress. Additionally, we show a new mechanism for microsatellite instability, double strand break formation. Classically, instability of microsatellite sequences was determined by the expansion or contraction in the number of (CTG)·(CAG) repeats (Fernandez-Lopez et al., 2004; McMurray, 2010; Zeman and Cimprich, 2014).

In addition to $(CTG)_{102}\cdot(CAG)_{102}$ repeats, we also examined the effect of loss of FANCJ on $(CTG)_{12}\cdot(CAG)_{12}$ repeats with the treatment of aphidicolin. Surprisingly, this normal repeat length was also unstable under these conditions. These results suggest FANCJ is necessary for the stability of all microsatellite sequences pathological and nonpathological increasing the importance in understanding the mechanism behind this phenomenon.

We observed a difference in sensitivity to FANCJ helicase depletion and aphidicolin treatment between $(CTG)_n$ and $(CAG)_n$ cells. Irrespective of (CTG)·(CAG) repeat length, $(CAG)_n$ cells were more sensitive to aphidicolin and FANCJ helicase depletion compared to $(CTG)_n$ cells. This observation has also been seen by other researchers in the laboratory. We believe this different in sensitivity leads to the variability in small pool PCR signal loss at the ectopic site $(CTG)_n\cdot(CAG)_n$ repeats between $(CAG)_n$ cells and $(CTG)_n$ cells.

FANCJ is essential for microsatellite stability across the genome

Trinucleotide repeats consisting of (CTG)·(CAG) sequences represent only one subset of microsatellite sequences. Other subtypes of microsatellites varying in length and nucleotide composition exist. Collectively, microsatellites make up approximately

3% of the entire genome. Surprisingly, multiple endogenous microsatellites showed instability with the depletion of FANCJ helicase and presence of aphidicolin or hydroxyurea in CAG_{102} cells. The same endogenous microsatellites were also unstable in FANCJ null fibroblast cells exposed to replication stress indicating microsatellite instability is not limited to (CTG)·(CAG) trinucleotide repeats but transpires at diverse microsatellite sequences. FANCJ null cells complemented with FANCJ wild-type cDNA rescued the phenotype of microsatellite instability under replication stress in FANCJ null cells solidifying a role for FANCJ helicase in microsatellite stabilization across the genome during replication stress. Previous work has shown that FANCJ null cells exhibit an increased level of chromosomal abnormalities when exposed to naturally occurring replication stress (Kuzminov, 2013; Noda et al., 2011), therefore, our work suggests diverse microsatellites are inherently prone to breakage and FANCJ is required for the prevention of double strand break formation during natural replication stress. Further, these results raise the question of whether there is a common intermediate, such as a stalled replication fork, which leads to double strand breaks. Additionally, our results suggest that microsatellite instability can be used as diagnostic tool to assay for FANCJ deficiency.

We noticed differences in the extent of small pool PCR signal loss between microsatellite sequences across the genome. The stability of secondary (non-B form) DNA structures varies (Bochman et al., 2012). We suspect the difference in stability between hairpins, triplexes, G-quadruplexes and other non-B form structures leads to differential small pool PCR signal loss.

Recently, a FANCJ helicase null mouse was created. Mouse embryonic fibroblasts (MEFs) from the knockout mouse model for FANCJ helicase show increased sensitivity to aphidicolin when compared to FANCJ proficient controls (Matsuzaki et al., 2015). Additionally, genomic instability is observed at a number of microsatellite sequences across the genome, homologous murine microsatellites sites to microsatellites sites we conveyed (Matsuzaki et al., 2015), confirming our results suggesting FANCJ is necessary for microsatellite stabilization during replication stress.

DSBs induced by depletion of FANCJ and replicative stress lead to G2/M arrest

Experiments presented in this work were conducted in the presence of a low dose of aphidicolin or hydroxyurea that does not acutely activate the DNA checkpoint response (Casper et al., 2002; Chen et al., 2015; Glover et al., 1984; Petermann et al., 2010; Wist, 1980). However, we examined the cell cycle profiles of FANCJ null cells and HeLa cells treated with siControl or siFANCJ exposed to aphidicolin and determined that the cells depleted of FANCJ are more sensitive to aphidicolin treatment. Additionally, FANCJ null cells treated with aphidicolin accumulated in the G2/M phase of the cell cycle indicative of a DNA checkpoint response. Previously published data of cell cycle profiles from normal fibroblast cells treated with aphidicolin show a slower progression through S phase indicative of aphidicolin affecting replication polymerases (Mao et al., 2008b). Here we show the additive loss of FANCJ helicase, in FANCJ null cells, leads to a different cell cycle profile, in which cells stall in the G2/M phase. Experiments are under way in the laboratory to assess the phosphorylation status of the DNA checkpoint kinase 1 (Chk1) and checkpoint kinase 2 (Chk2) as well as the kinases

upstream of Chk1 and Chk2, Ataxia telangiectasia mutated (ATM) and Ataxia telangiectasia and Rad3 related kinase (ATR) to understand the mechanism of checkpoint activation in cells depleted of FANCJ under replication stress.

FANCJ functions independently of the FA repair pathway

FANCJ is the helicase associated with the FA repair pathway. Activation of the FA repair pathway occurs in response to replication stress and DNA damage. The requirement of FA repair pathway activation for FANCJ to stabilize microsatellites was examined. Knockdown of FANCM and FANCD2 in $(CTG)_{102}\cdot(CAG)_{102}$ DM1 model cells exposed to replicative stress does not lead to instability of ectopic site (CTG)·(CAG) repeats or endogenous microsatellites suggesting FANCJ functions independently of the FA repair pathway to maintain microsatellite stability. FANC null patient cells treated with either aphidicolin or hydroxyurea to induce replication stress show a similar pattern of instability at all endogenous microsatellites analyzed confirming FANCJ helicase stabilizes microsatellites irrespective of activation of the FA repair pathway. A concern of the experimental approach may be that instability is occurring and goes undetected by our end-point PCR approach. Endogenous sites and the ectopic site were examined in multiple FANC null protein conditions both with and without replicative stress. Small pool PCR results under all combination of conditions (different FANC protein null cells treated with aphidicolin or hydroxyurea) did not show variability in results, no loss in spPCR signal. If instability was taking place, it is likely that we would have observed inconsistencies in small pool PCR results. In addition, we performed a small pool PCR titration experiment in which we varied the ratio of copy

number between the ectopic site and an internal control site. It was evident that loss of PCR signal is detectable via our end-point small pool PCR method.

Recently, a number of FANC proteins have been implicated in DNA replication and repair processes independent of the FA repair pathway, as we have shown for FANCJ. To begin, FANCM functions with associated protein FAAP24 to recognize DNA damage and mediate the Ataxia telangiectasia and Rad3 related kinase (ATR)/ checkpoint kinase 1 (Chk1) checkpoint response to prevent accumulation of unrepaired DNA damage independent of the FA core complex (Collis et al., 2008; Schwab et al., 2010; Wang et al., 2013). In addition, ATR-mediated replication fork restart is deficient in FANCM null cells when compared to other FANC null cells (FANCC and FANCI) (Schwab et al., 2010). Phosphorylation of FANCI by ATR in response to replication stress leads to dormant origin firing and replication fork restart (Chen et al., 2015). FANCD2 functions with BLM helicase to promote replication fork recovery independently of FANCI and the FA pathway (Chaudhury et al., 2013). Most recently, FANCD2, FANCD1/BRCA2, and FANCJ have been implicated in replication fork recovery after treatment with aphidicolin or hydroxyurea independently of the FA core complex (Raghunandan et al., 2015). Taken together, it is not surprising FANCJ functions irrespective of FA repair pathway activation. FANCM, FANCI, FANCD2, and FANCD1/BRCA2 depleted or null cells were treated with aphidicolin and hydroxyurea. Microsatellite instability was not detected under these conditions suggesting the FA independent roles of other FANC proteins are also not essential for FANCJ to stabilize microsatellites further confirming a uniquely novel function for FANCJ helicase in the protection of microsatellite integrity.

Coupled with recent work from MEFs from FANCJ helicase null mice (Matsuzaki et al., 2015), our results suggest FANCJ helicase functions independently of the FA repair pathway to stabilize microsatellite sequences. We suspect FANCJ functions in different DNA repair processes based upon interaction with different repair proteins. For function in the FA repair pathway FANCJ requires helicase activity and interaction with BRCA1 (phosphorylation at serine 990). However, for stabilization of microsatellites, FANCJ does not rely on activation of the FA repair pathway.

Translocations occur in FANCJ depleted cells during replicative stress

$(CTG)_{102}\cdot(CAG)_{102}$ DM1 model cells depleted of FANCJ helicase and treated with aphidicolin resulted in (CTG)·(CAG) trinucleotide repeat stability. Interestingly, sequences 1-3kb from the (CTG)·(CAG) repeats of the ectopic site remained intact while primers <100 bp from the repeats did not give a PCR product. These data suggested the primer binding sites used for spPCR are no longer in close proximity to one another, which would result from a double strand break (DSB). To determine if double strand breaks lead to translocations and where those translocations are located, we employed an inverse PCR (iPCR) approach. Long distance iPCR has previously been used to identify translocations in cancer cells and many reports have discussed importance of iPCR approaches to determine unknown translocation sites (Benkel and Fong, 1996; Ochman et al., 1988; Schneider et al., 2011) supporting iPCR as a good technique to determine translocations at the ectopic site. Coupling iPCR with next generation sequencing (Meier and Gartner, 2014) proved to be the ideal application. Inverse PCR results revealed that translocations occurred between the ectopic site (CTG)·(CAG) repeats and another genomic site. Translocation sites determined by iPCR have been identified

cytogenetically. The cytogenetic positions of all the translocation sites we have identified have previously been associated with chromosome instability, cancer, and developmental disorders. Chromosome 2p22.3 is linked to small cell lung carcinoma (Lui et al., 2001), sesorineural nonsyndromic recessive deafness (Chaib et al., 1996), and Maffucci syndrome (development of multiple enchondromas associated with hemangiomas, predisposed to CNS, pancreatic, and ovarian malignancies) (Amyere et al., 2014). In addition, chr. 2q12.2 is associated with small cell lung carcinoma (Lui et al., 2001) and chr. 2q34 with breast cancer (Kim et al., 2012) and Bjornstad syndrome (developmental disorder associated with deafness) (Lubianca Neto et al., 1998). Ovarian cancer (Kamieniak et al., 2015; Wan et al., 1999), breast cancer (Li et al., 2007), neuroblastoma (Li et al., 2007), and melanoma (Li et al., 2007) have been linked to chr. 6q24.1. Chromosome 7p15.3 is connected with multiple disorders including Dandy Walker syndrome (brain malformations) (Chen et al., 2010), hand-foot-genital syndrome (limb malformations and urogenital abnormalities) (Hosoki et al., 2012), myeloma (Broderick et al., 2012; Martino et al., 2012; Weinhold et al., 2015), childhood lymphoma/leukemia (Kaneko et al., 1988) and ovarian cancer (Li et al., 2014; Shimada et al., 2013). Chromosome 8q24.21 associates with gliomas (Enciso-Mora et al., 2013; Lu et al., 2015), renal cell carcinoma (Gudmundsson et al., 2013), male breast cancer (Silvestri et al., 2015), lymphoma (Enciso-Mora et al., 2010), and T-cell leukemia (Crowther-Swanepoel et al., 2010). Chromosome 10q22.3 is linked to Cowden disease (growth of multiple hamartomas and increased risk of cancer) (Nelen et al., 1996), breast cancer (Chen et al., 2012; Kiyotani et al., 2012), ovarian cancer (Ho et al., 2009), non-small cell lung cancer (Petersen et al., 1998; Yendamuri et al., 2008), and prostate cancer (Lu and Hano, 2008;

Pan et al., 2001). Chromosome 11p15.4-5 relates to breast cancer (Wikman et al., 2012), Silver-Russell syndrome (delayed growth rate before and after birth) (Demars and Gicquel, 2012; Eggermann et al., 2010), Beckwith Wiedemann syndrome (entire body developmental abnormalities coupled with cancer predisposition) (Baskin et al., 2014; Demars and Gicquel, 2012; Demars et al., 2011), and T lymphoid leukemia (Crescenzi et al., 2015). Hepatocellular carcinoma (Wang et al., 2015b) and colorectal cancer (Houlston et al., 2010) have been linked to chr. 20q13.13. We also note with interest that a majority of the 300-500bp translocation site reads had a sequence predicted to form noncanonical DNA structures, and are prone to double strand breaks in the absence of FANCJ during replication stress from our studies, near the translocation junction (2p22.3 – G_{300}; 2q12.2 – G4; 2q34 – G4; 7p15.3 – G4; 8q24.21 – G4; 11p15.4 – G4; 20q13.13 – G4; 6q24.1 – T_{20}; 19p13.32 – CTG_{20}). We predict that DSBs form at microsatellite sequences resulting in pools of free DNA ends which are substrates for classical DNA repair pathways including non-homologous ending joining and microhomology-mediated end joining, or form structures which cannot be resolved by classical DNA repair processes. The high frequency of microsatellite DSBs in FANCJ depleted and null cells during replication stress prompts us to consider that inherited or stochastic FANCJ deficiency may pose a threat to proper replication of sequences known to form noncanonical DNA structures resulting in chromosome aberrations. As a protector of microsatellites, FANCJ possibly participates in the prevention of cancer and developmental disorders broadening current understanding of FANCJ function.

Our data show double strand break formation at microsatellite sequences in cells depleted of FANCJ helicase under replicative stress. The approach used in our

experiments analyzes the repair of structure induced DNA breaks (double strand breaks resulting from the presence of noncanonical DNA structures). The most common method for identifying repair proteins and processes involved in the repair of double strand breaks occurs through utilization of an I-Sce1 endonuclease restriction site (Choulika et al., 1995; Liang et al., 1998). I-Sce1 recognizes and cuts DNA at an unique 18bp sequence not found in the human genome (Monteilhet et al., 1990). An I-Sce1 expression vector is co-transfected into cells with a plasmid containing a selectable marker that will only express if homologous recombination occurs after I-Sce1 cleavage (Liang et al., 1998). The level of selectable marker expression (fluorescence protein or resistance gene) is measured and correlates to the amount of double strand breaks repaired by homologous recombination (Liang et al., 1998). Coupling this technique with the knockdown of replication and repair proteins has identified many proteins that participate in homologous recombination (Johnson et al., 1999; Liang et al., 1998; Pierce et al., 1999). A similar system has been created to analyze nonhomologous end joining through I-Sce1 double strand break formation (Mao et al., 2008a). However, these approaches only identify proteins involved in the repair of a controlled, direct double strand break produced by I-Sce1 cleavage. Only recently has structure induced breaks been examined. Carboxyterminal binding protein-interacting protein (CtIP) has been identified in the protection of common fragile sites and inverted repeat stability (Wang et al., 2014). This report used an I-Sce1 experimental design, in which a structure forming sequence was placed adjacent to the I-Sce1 cut site and noncanonical structure formation occurs after cleavage by I-Sce1 (Wang et al., 2014). In comparison, our data show FANCJ helicase is necessary in the prevention of structure induced double strand breaks. Additionally, the

structure induced breaks naturally occur in our model, unlike controlled, direct breaks created from I-Sce1 cleavage. Therefore, our approach offers a new method for identifying replication and repair proteins essential for the resolution of structure induced double strand breaks.

A large majority of next generation sequencing reads from iPCR containing translocation sites mapped to the endogenous c-myc locus at chr. 8q24.21. The ectopic site is located on chromosome 18 (Liu et al., 2003). Translocations from the ectopic site c-myc core replicator to the endogenous c-myc locus occurred via non-allelic homologous recombination. Coupled with the presence of microsatellite sequences at other translocation sites, we propose that repair of DSBs formed at microsatellites is nonrandom. However, it is possible that we are promoting nonrandom recombination with the presence of the c-myc core replicator at the ectopic site. Further experiments are underway to characterize translocations due to breaks at endogenous sites in a cell line void of ectopic homologous sequences. For instance, restriction digestion, ligation and iPCR followed by next generation sequencing of an endogenous microsatellite (i.e. (CTG)·(CAG) repeats of the DMPK locus or ATTCT repeats of the SCA10 locus) in FANCJ null cells treated with aphidicolin would be a good model. Results from these experiments would determine if translocations occurring from DSBs at microsatellite sequences show a similar propensity to repair the DSBs followed by translocation in an orderly fashion via a subtype of recombination, or if we have exploited the model and forced nonrandom recombination. Determining if the patterns of repair are different at endogenous sites compared to the ectopic site is the goal. Additionally, we will determine if the ectopic site (CTG)·(CAG) breaks induced breaks at endogenous microsatellite, or if

endogenous microsatellite sequences break in the absence of FANCJ during replication stress leaving a pool of broken DNA ends for repair by non-allelic homologous recombination or microhomology-mediated end joining. Nonrandom repair would result in translocations sites similar to the sites obtained from iPCR and NGS of the ectopic (CTG)·(CAG) repeats. However, if a pool of DNA ends are created as a result of replication stress in FANCJ null cells at microsatellite sequences and repair occurs through microhomology-mediated end joining, we would expect the translocations sites to differ between the ectopic site (CTG)·(CAG) repeats and the endogenous ATTCT repeats of the SCA10 locus (or any other endogenous microsatellite sequence).

Lastly, the next generation sequencing data presented here represents only a small subset of data obtained from the sequencer. The nucleotide positions of each end of the reads (the first nucleotide and the last nucleotide of the read) containing translocation junctions were used to identify the chromosome sites of translocations (as well as cytogenetic positions). However, the exact breakpoint junctions were not identified. Software exists to process deep sequencing data sets for breakpoint junctions including Bellerophon (Hayes and Li, 2013), and we are currently using this software to narrow the breakpoint junction sequences and determine whether translocation induces error-prone break induced replication (BIR). Recently, computer software, SVDetect (Zeitouni et al., 2010), was created to recognize genomic variations in the sequencing data. Analysis of the sequencing data with Bellerophon and SVDetect, should provide insights into breakpoint junction locations as well as mutations that arose during the transition of double strand breaks to fused translocations.

Microsatellite stabilization by FANCJ helicase is replication dependent

We have shown here a novel function for FANCJ helicase in microsatellite stabilization during replicative stress. This novel function of FANCJ helicase is independent of activation of the FA repair pathway. The microsatellite sequences examined in this work vary in accessibility of proteins to chromatin. Some sequences are replicated and transcribed increasing the length of time the DNA is exposed and other sequences are not transcribed in HeLa cells, such as β-globin (Green et al., 1983). We suspect small pool PCR signal loss across genome is a function of the entire genome being replicated during the cell cycle supporting the notion that this novel function of FANCJ helicase is replication dependent and independent of other cellular processes such as transcription.

Replication fork regression vs. replication fork stalling

Our results identify a novel function for the FANCJ helicase in microsatellite stabilization during replication stress. We have shown that double strand breaks form in cells depleted of FANCJ under replicative stress. However, when the double strand break occurs still remains unknown. The double strand break could form as a result of inability to resolve a noncanonical DNA structure (London et al., 2008) at the microsatellite sequence in the absence of FANCJ or through defective DNA repair mechanisms, since FANCJ functions in replication fork regression (Brosh, 2013; Neelsen and Lopes, 2015; Zellweger et al., 2015) and enables homologous recombination (Litman et al., 2005). Understanding the mechanisms and identifying the proteins involved in supporting FANCJ helicase to stabilize microsatellite sequences to protect genome stability is of increasing interest. We would like to deplete proteins whose functions are specific to fork

regression or homologous recombination in the presence of replication stress and examine microsatellite stability to determine the proteins functioning in signaling cascades necessary for FANCJ helicase to prevent microsatellite instability.

Additionally, we propose to examine FANCJ helicase activity in replication fork regression and replication fork stalling via density-gradient separation of bromodeoxyuridine (BrdU) labelled DNA coupled with chromatin immunoprecipitation (ChIP). BrdU labelled DNA strands are heavier than that of unlabeled DNA strands (Painter and Cleaver, 1969). BrdU is incorporated into nascent DNA strands during DNA replication creating labelled DNA. Unlabeled DNA strands are referred to as light strands and BrdU labelled DNA strands are termed heavy strands. Possible combinations double strand DNA comprised of light strands and heavy strands have the following densities: light - light strand has the density of 1.716 to 1.722 g/cm^3, heavy - light strands density are between 1.755 to 1.761 g/cm^3, and heavy - heavy strands have a density between 1.821 to 1.831 g/cm^3 (Painter and Cleaver, 1969). Coupling BrdU labelling of DNA with ChIP specifies the DNA sequences of interest. Pulldown of FANCJ helicase after BrdU labelling would reveal only the sequences linked to FANCJ helicase (DNA sequences of interest) while discarding other DNA sequences. Reversal of crosslinking between DNA to protein, frees the DNA sequences for separation. A stalled replication fork would contain light-light or heavy-light DNA (Figure 63). However, replication fork regression occurs in response to DNA damage in which replication is reversed resulting in re-replication of the nascent strand DNA producing heavy-heavy DNA (Figure 63). Comparing the composition of DNA pulled down with FANCJ during replication stress will provide insights into the presence of FANCJ helicase at stalled replication forks or

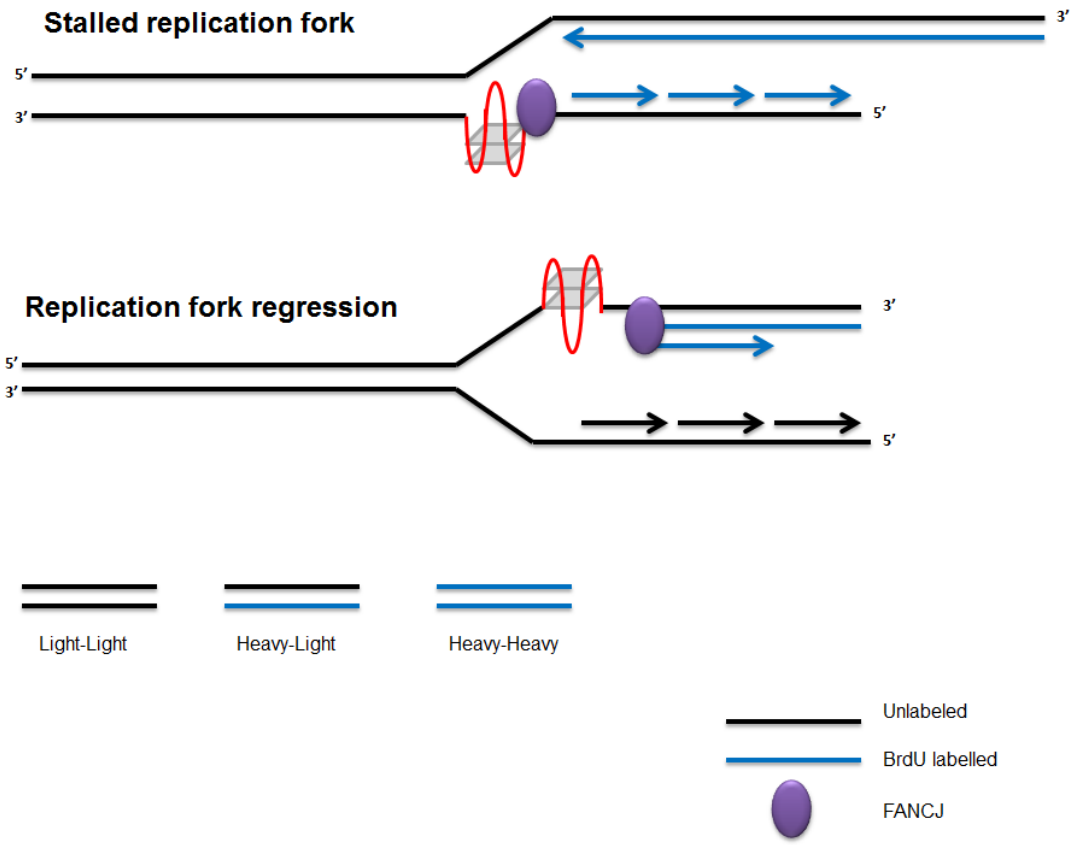

Figure 63. Proposed roles for FANCJ in replication fork stalling and regression. FANCJ helicase may function in either replication fork stalling or replication fork regression. Utilizing BrdU to label nascent strand DNA during replication stress with help determine which process FANCJ functions in and if these processes occur at microsatellite sequences.

regressed replication forks. For additional comparison, DNA sequences pulled down with BRCA2 will serve as an example for replication fork stalling, since BRCA2 has been shown to protect replication forks from stalling previously (Lomonosov et al., 2003; Schlacher et al., 2011; Ying et al., 2012). After separation of labelled and unlabeled DNA sequences by density, individual groups of DNA sequences (light-light, heavy-light or heavy-heavy) could be subjected to quantitative PCR (qPCR) to determine the abundance of microsatellite sequences present in each category. A greater abundance of microsatellite sequences in either heavy-light or heavy-heavy DNA depending on the process, replication fork stalling or replication fork regression respectively, disrupted in microsatellite instability in the absence of FANCJ helicase during replicative stress is expected.

FANCJ stabilizes microsatellites independent of BLM/WRN activity

Current models of genome stabilization by helicases whose dysfunction leads to a genome instability disorder suggest a cooperative mechanism in which FANCJ unwinds noncanonical DNA structures in a 5’ to 3’ orientation while BLM helicase or WRN helicase unwind non-B form structures in a 3’ to 5’ orientation. Function of both direction specific helicases is necessary for proper structure resolution leading to error free DNA replication and repair. Knockdown of BLM helicase in $(CTG)_{102}\cdot(CAG)_{102}$ DM1 model cells under replication stress did not produce microsatellite instability suggesting the activity of BLM helicase is nonessential for FANCJ to maintain microsatellite stability. Similar results were obtained in BLM helicase null patient cells treated with either aphidicolin or hydroxyurea suggesting indeed FANCJ stabilizes microsatellites independently of BLM helicase activity. Furthermore, the treatment of a

Fanconi anemia patient cell harboring the homozygous FANCJ^{R798X} mutation eliminating BLM helicase interaction with aphidicolin or hydroxyurea also did not result in microsatellite instability further confirming BLM function remains dispensable in microsatellite stabilization by FANCJ helicase.

Knockdown of WRN helicase in $(CTG)_{102}\cdot(CAG)_{102}$ DM1 model cells exposed to aphidicolin showed no instability at microsatellites indicating WRN activity is nonessential for FANCJ helicase to maintain chromosome stability. WRN null cells treated with either aphidicolin or hydroxyurea confirmed results depicting no microsatellite instability further confirming FANCJ helicase functions to stabilize microsatellites irrespective of WRN helicase activity. Considering the data presented regarding depletion of BLM helicase and WRN helicase, our data suggest an unique function for FANCJ helicase separating FANCJ from previously proposed models of cooperative activity with BLM helicase and WRN helicase.

BLM helicase and WRN helicase belong to the RecQ family of helicases along with RECQL4 (associated with Rothmund Thomson syndrome) (Kitao et al., 1999; Nakayama, 2002). Recent work has indicated redundancy exists in the function of this family of helicases (Sidorova et al., 2013; Sturzenegger et al., 2014). BLM helicase and WRN helicase function additively promotes replication fork progression (Sidorova et al., 2013). Additionally, WRN helicase can substitute for BLM helicase during the DNA damage response for DNA end resection catalyzed by DNA2 (Sturzenegger et al., 2014). Here, we have examined the loss of BLM helicase and WRN helicase during replicative independently. It would be interesting to knockdown BLM helicase and WRN helicase simultaneously during replication stress, since these redundancies exist within the RECQ

family of helicases. However, we suspect no microsatellite instability would be observed similar to the loss of BLM helicase and WRN helicase individually. Initially, microsatellite stabilization during replication stress appeared to be 5' to 3' helicase specific.

FANCJ-like family of DNA helicases

Based on our data, we suspected microsatellite stability during replication stress relies on the activity of a 5' to 3' unwinding enzyme irrespective of 3' to 5' unwinding helicase activity. However, this work only examines the loss of FANCJ on microsatellite stabilization during replicative stress. A new family of helicases has emerged with sequence homology to FANCJ helicase (Wu et al., 2009). Helicases of this FANCJ-like family include XPD, ChiR1/DDX11, and RTEL1 (Wu et al., 2009). All members of this family of helicases share a conserved helicase core domain (Wu et al., 2009) (Figure 64). XPD helicase functions in nucleotide excision repair (Fan et al., 2008). ChiR1/DDX11 helicase promotes sister chromatin cohesion (Wu et al., 2012). Lastly, the helicase RTEL1 helicase unwinds noncanonical DNA structures including G-quadruplexes formed at telomeres, is essential for trinucleotide repeat integrity, and is thought to promote genome stability in cancer cells through the suppression of homologous recombination (Barber et al., 2008; Frizzell et al., 2014; Uringa et al., 2011; Vannier et al., 2014). Redundancy between the functions of these helicases may exist due to sequence homology of the catalytic helicase domain. It is likely these helicases have similar function to FANCJ helicase in response to replicative stress. We suspect the loss of XPD, ChiR1/DDX11, or RTEL1 coupled with replication stress would lead to microsatellite instability as seen with the loss of FANCJ helicase. We propose that

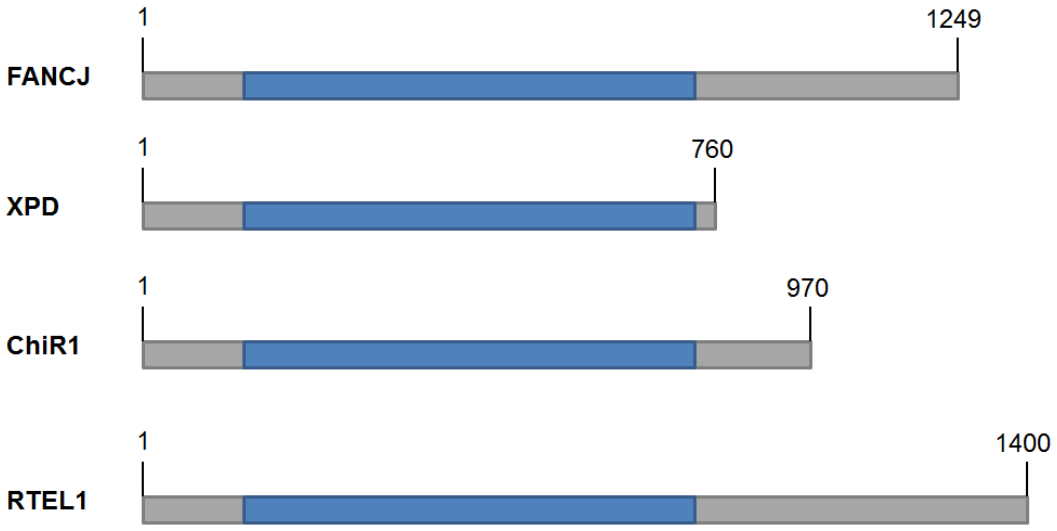

Figure 64. FANCJ-like family of helicases. The FANC-like family of DNA helicases includes XPD, ChiR1, and RTEL1. A conserved helicase core domain is shared among all family members (blue bar). (Adapted from Wu et al., 2009)

microsatellite stabilization during replicative stress is a function of the FANCJ-like family of helicases.

5’ to 3’ unwinding DNA helicases similar to FANCJ helicase

Other 5’ to 3’ unwinding helicases that do not belong to the FANCJ-like family of helicases exist including Pif1 and Fbh1. Pif1 has been implicated in promoting the stability of G4 DNA, G-quadruplex forming DNA, at telomeres in human cells (Paeschke et al., 2011; Sanders, 2010). In addition, evidence exists to suggest Pif1 helicase function is important for resolving intermediates formed during replication and G-quadruplex structures formed at a guanine rich sequences at the HECT and RLD domain containing E3 ubiquitin protein ligase 5 (CEB1) locus (Paeschke et al., 2011; Pike et al., 2010; Ribeyre et al., 2009). Recent reports indicate the helicase Fbh1 localizes at DNA damage sites in response to accumulation of single strand DNA to promote replication fork regression and homologous recombination repair of DNA lesions (Chu et al., 2015; Fugger et al., 2009; Fugger et al., 2015). Since Pif1 and Fbh1 possess roles in DNA replication and repair similar to FANCJ helicase, it is possible these helicases also function in microsatellite stabilization during replication stress. We suspected microsatellite stabilization under replicative stress is a function of 5’ to 3’ DNA helicases who function in DNA replication or repair due to overlap in function between FANCJ and Pif1 or Fbh1. However, treatment of cells harboring a biallelic FANCJ^{R798X} mutation, which renders the protein helicase dead *in vitro*, with replication stressors aphidicolin or hydroxyurea revealed no loss of small pool PCR signal suggesting the helicase function of FANCJ is dispensable for microsatellite stabilization. Therefore, protection of microsatellite stability during replication stress is likely not a function of all 5’ to 3’

helicases and is specific to FANCJ helicase and possibly other members of the FANCJ-like family of helicases.

FANCJ^{R798X} mutant offers insights into functional domains of FANCJ

The most commonly occurring FANCJ mutation is R798X which produces a premature stop codon on the FANCJ transcript subsequently truncating the protein. This truncation eliminates binding sites for BRCA1, TopBP1 and BLM. Additionally, *in vitro* analysis of FANCJ^{R798X} indicated a loss of helicase activity. We examined the effect of truncating FANCJ by treating a Fanconi anemia complementation group J patient cell line harboring a homozygous FANCJ^{R798X} mutation with aphidicolin or hydroxyurea. Interestingly, microsatellite instability was not seen under these conditions. These results suggest BRCA1, TopBP1 and BLM binding or FANCJ helicase activity are not essential for FANCJ to maintain microsatellite stabilization. FANCJ interacts with other proteins necessary for DNA replication and DNA damage responses including Mre11 and the MRN complex (Suhasini et al., 2013), RPA (Gupta et al., 2007) and MLH1 (Peng et al., 2007). Recently, proteins containing iron-sulfur (Fe-S) clusters are of interest. The Fe-S cluster of multiple proteins has been implicated in genome stability through function in mitochondrial respiration and DNA repair (Beilschmidt and Puccio, 2014; Paul and Lill, 2014). FANCJ has a Fe-S cluster and this domain is essential for helicase activity (Rudolf et al., 2006; White, 2009). These domains, which are present in the truncated FANCJ^{R798X} mutant, may be essential for FANCJ to function in stabilization of microsatellites.

Several groups have published on the inability to detect the FANCJ^{R798X} truncated mutant *in vivo* (Cantor and Guillemette, 2011; Litman et al., 2005). Experiments are

underway in our laboratory to create an epitope tagged FANCJ^{R798X} mutant protein for the detection of the mutant protein via Western blot, and examine its *in vivo* function. Expression of a tagged version of FANCJ^{R798X} in FANCJ null cells could be used in rescue experiments to determine if the domains present in the truncated protein are sufficient for FANCJ to stabilize microsatellites. We suspect these experiments will offer insights into the mechanism behind FANCJ function in promoting microsatellite stability.

As previously stated, recombinant FANCJ^{R798X} helicase does not retain helicase activity *in vitro*. However, it is unclear if FANCJ^{R798X} helicase remains inactive *in vivo*. To confirm helicase function is dispensable for FANCJ to maintain microsatellite stability, mutation of lysine 52 to arginine on FANCJ (Cantor et al., 2001) could be performed. This residue is critical for ATPase hydrolysis and mutation leads to dysfunction of helicase activity (Cantor et al., 2004; Cantor et al., 2001; Gupta et al., 2005). Expression of FANCJ^{K52R} in FANCJ null cells under replication stress would aid in understanding the role the helicase domain has in microsatellite stabilization. If it is determined that the helicase activity of FANCJ is not essential for microsatellite stability, it is likely FANCJ functions as a signal for the recruitment of other repair proteins. Additionally, FANCJ-like helicase family members who have conserved regions of homology may also function as a signaling molecular similar to FANCJ helicase; therefore, the loss of these helicases during replication stress is of interest as well.

As previously stated, FANCJ helicase may function in different repair processes. In the FA repair pathway, FANCJ utilizes interaction with BRCA1 and helicase function to aid DNA repair. Here, we show a second cellular process FANCJ functions in, microsatellite stabilization during replication stress. For microsatellite stabilization,

FANCJ function independent of interactions with BRCA1, TopBP1 and BLM helicase, activation of the FA repair pathway, and additionally, helicase activity is unessential.

Possible mechanisms for FANCJ mediated microsatellite stabilization

This work has not focused on a mechanism of action for FANCJ helicase in microsatellite stabilization. However, we have shown functional domains not essential for is this function of FANCJ helicase (i.e. BRCA1, TopBP1, and BLM helicase interaction). Therefore, we propose three possible mechanisms of action: (i) The FANCJ^{R798X} truncation mutations of FANCJ retains interaction sites for MLH1 and interaction with MLH1 and other mismatch repair proteins may lead to the recruitment of FANCJ to microsatellite sequences. (ii) Previously published work from other researchers has identified interaction of Mre11 and RPA with FANCJ. However, the domains necessary for these interactions on FANCJ are unknown. It is possible that FANCJ is utilizing interaction with Mre11 or RPA for recruitment to the site of DNA damage. (iii) It is possible that FANCJ helicase is traveling with the replication fork during replication through an unknown interaction with the replisome. Recently, it was determined that RTEL1, a FANCJ-like helicase family member, directly interaction with proliferating cell nuclear antigen (PCNA) during replication and promotes proper DNA replication, replication stability, and telomere stability (Vannier et al., 2013). Therefore, it is possible FANCJ also interacts with the replisome. To understand the mechanism of action for FANCJ helicase in microsatellite stabilization during replication stress, these avenues must be examined. First, mutation of MLH1 binding domains on FANCJ would identify the necessity for MLH1 binding. Second, identification of Mre11 and RPA binding sites on FANCJ would add insight into the dependency on Mre11 and RPA interaction for

FANCJ to stabilize microsatellite sequences. Lastly, immunoprecipitation followed analysis with mass spectroscopy would determine other possible unknown interactions between FANCJ and other replication and repair proteins offering insights into the mechanism behind microsatellite stability by FANCJ helicase.

Dog-1 as an alternate to FANCJ helicase in microsatellite stabilization

The *C. elegans* orthlog of FANCJ, dog-1 (Youds et al., 2008), has previously been associated with the stabilization of G4 DNA sequences (thought to fold into the non-B form structure G-quadruplex) (Cheung et al., 2002; Kruisselbrink et al., 2008). Comparison of amino acid sequences of FANCJ and dog-1 revealed a conserved helicase domain between both proteins (Figure 65). In addition, the C-terminus of the FANCJ is not present in dog-1. The C-terminus includes interaction sites with BLM helicase, BRCA1, and TopBP1. These domains are eliminated in the truncation, R798X, mutation of FANCJ. Interestingly, this mutation was sufficient to protect against microsatellite instability. These results concur with results from experiments examining dog-1. Full length dog-1 (the same domains are present in the R798X mutation of FANCJ) stabilizes G4 DNA sequences. However, loss of dog-1, comparable to FANCJ null cells void of FANCJ, shows deletions of sequences flanking G4 DNA supporting the results we have shown confirming a role for FANCJ helicase in microsatellite stability. We hypothesize that expression of dog-1 in FANCJ null cells would be adequate to rescue microsatellite instability during replication stress. Additionally, mutation of other domains still present in the $FANCJ^{R798X}$ would lead to identification of functional domains necessary for microsatellite stabilization.

Figure 65. Amino acid comparison of FANCJ and dog-1. A conserved helicase core domain (blue bar) is present in both human FANCJ helicase and the *C. elegans* ortholog, dog-1. (Adapted from Wu et al., 2009)

FANCJ helicase function in cancer

Recent work has identified a variety of separation of function FANCJ mutations. Figure 66 depicts a schematic of FANCJ mutations associated with breast cancer only, Fanconi anemia only, or both breast cancer and Fanconi anemia supporting distinctly different roles for FANCJ associated with a diverse variety of pathologies from cancer to developmental disorders (Cantor and Guillemette, 2011). It is likely that inability of FANCJ to stabilize microsatellites accompanies a subset of FANCJ mutations. Examining additional pathological FANCJ mutations is of interest to separate function in the FA repair pathway from the protection of microsatellite stability.

FANCJ helicase as a cancer therapeutic target

In addition to Fanconi anemia, mutation or loss of FANCJ helicase has been linked to susceptibility of multiple cancers including breast cancer (Cantor and Guillemette, 2011; De Nicolo et al., 2008; Eelen et al., 2008; Guenard et al., 2007), ovarian cancer (Kuusisto et al., 2011; Rafnar et al., 2011), colon cancer (Nakanishi et al., 2012; Xie et al., 2010), and prostate cancer (Kote-Jarai et al., 2009). Multiple groups have referred to FANCJ helicase as a genomic caretaker and tumor suppressor (Cantor and Guillemette, 2011; De Nicolo et al., 2008; Peng et al., 2007; Wu et al., 2008). We believe our findings offer an advantageous approach to the development of new therapies to combat FANCJ helicase associated disorders. We suspect FANCJ helicase is a potential therapeutic target for the treatment of many cancers. A combinational approach could be developed in which FANCJ inhibition occurs during treatment with replication stress inducing reagents to produce genome instability. Small molecule inhibitors could be used to block FANCJ function followed by treatment with replication inhibitors to

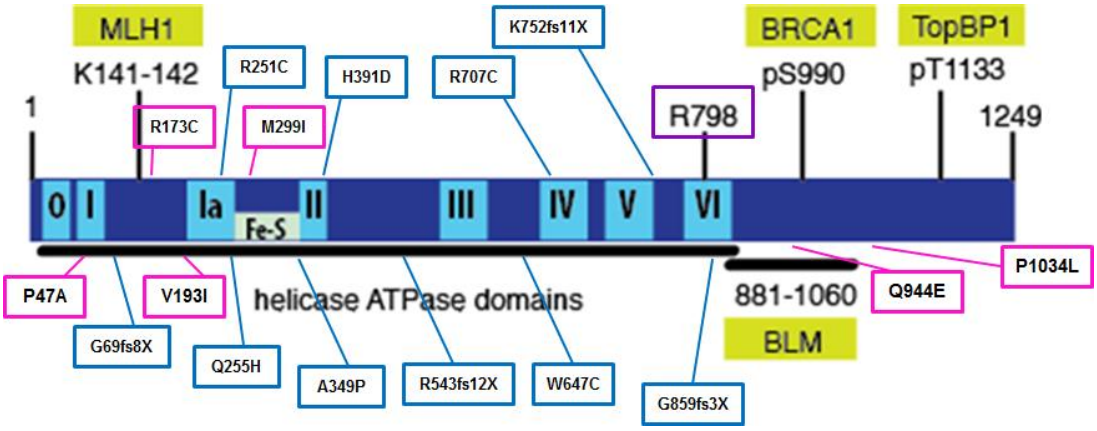

Figure 66. Schematic of FANCJ mutations associated with disease. Mutations in FANCJ that are linked to breast cancer (blue), Fanconi anemia (pink) or breast cancer and Fanconi anemia (purple). (Adapted from Cantor and Guillementte, 2011)

slow the growth of tumors. Recently, a small molecule inhibitor was created to inhibit the activity of WRN helicase (Aggarwal et al., 2011). Treatment of HeLa cells with the small molecular inhibitor resulted in increased DNA damage and inhibition of cell proliferation (Aggarwal et al., 2011). Additionally, a recent report showed that decreased levels of FANCJ sensitized gastric cancer cells to oxalipalatin (platinum based chemotherapeutic) (Mori et al., 2013) supporting our hypothesis of combinational therapy to damage and ultimately destroy cancer cells. Coupling the loss of FANCJ function (through gene therapy or small molecule inhibitors) with a DNA damaging drug could be a novel therapeutic approach for the treatment of FANCJ associated cancers (breast, ovarian, prostate, and colon).

FUTURE DIRECTIONS

The work presented in this dissertation identifies a novel function for the FANCJ helicase in microsatellite stabilization during replication stress. To move forward with the project, we are currently creating epitope tagged expression plasmids for wild-type and mutant FANCJ helicase. The mutant constructs include disruption of MLH1 binding, the $FANCJ^{R798X}$ mutation to determine if a truncated protein is expressed *in vivo*, and a catalytically inactive helicase domain created by a point mutation at lysine 52. The goal of mutagenizing FANCJ helicase focuses on determining the mechanism of action for FANCJ helicase in microsatellite stabilization. Additionally, we are performing endonuclease digestion, intramolecular ligation and inverse PCR at an endogenous microsatellite sequence ($(CTG)_{19}$ repeats of the DMPK locus and $(ATTCT)_{13}$ repeats of the SCA10 locus) in FANCJ null cells treated with aphidicolin. We are interested in understanding the mechanisms behind repair of double strand breaks at microsatellite sequences in cells depleted of FANCJ helicase under replicative stress. We are determining the phosphorylation status of checkpoint kinases including ATR, ATM, Chk1, and Chk2 to determine the mechanism of G2/M accumulation over time during aphidicolin treatment in FANCJ null cells. Lastly, we are using Bellerophon software to determine the exact breakpoint junctions from the inverse PCR next generation sequencing data. In addition, SVDetect will be used to understand the mechanisms used for repair and if repair occurred in an error-free process.

CONCLUSION

The work presented in this document focuses on identifying a novel function for the FANCJ helicase in stabilization of microsatellites. Stabilization of microsatellites by FANCJ occurs independently of the FA repair pathway and BLM helicase/WRN helicase activity. Double strand breaks form at the microsatellites resulting in translocations in FANCJ depleted cells during replication stress. Inverse PCR coupled with next generation sequencing provided a link between FANCJ function in microsatellite stability and a variety of cancers and developmental diseases suggesting a role for FANCJ in other disorders aside from Fanconi anemia. These data expand the current model of FANCJ function while opening a new avenue of diagnostic tool development for FANCJ insufficiency.

REFERENCES

Aggarwal, M., Banerjee, T., Sommers, J.A., Iannascoli, C., Pichierri, P., Shoemaker, R.H., and Brosh, R.M., Jr. (2013). Werner syndrome helicase has a critical role in DNA damage responses in the absence of a functional fanconi anemia pathway. Cancer research *73*, 5497-5507.

Aggarwal, M., Sommers, J.A., Shoemaker, R.H., and Brosh, R.M., Jr. (2011). Inhibition of helicase activity by a small molecule impairs Werner syndrome helicase (WRN) function in the cellular response to DNA damage or replication stress. Proceedings of the National Academy of Sciences of the United States of America *108*, 1525-1530.

Aguilera, A., and Gomez-Gonzalez, B. (2008). Genome instability: a mechanistic view of its causes and consequences. Nature reviews. Genetics *9*, 204-217.

Amyere, M., Dompmartin, A., Wouters, V., Enjolras, O., Kaitila, I., Docquier, P.L., Godfraind, C., Mulliken, J.B., Boon, L.M., and Vikkula, M. (2014). Common somatic alterations identified in maffucci syndrome by molecular karyotyping. Mol Syndromol *5*, 259-267.

Barber, L.J., Youds, J.L., Ward, J.D., McIlwraith, M.J., O'Neil, N.J., Petalcorin, M.I., Martin, J.S., Collis, S.J., Cantor, S.B., Auclair, M., *et al.* (2008). RTEL1 maintains genomic stability by suppressing homologous recombination. Cell *135*, 261-271.

Baskin, B., Choufani, S., Chen, Y.A., Shuman, C., Parkinson, N., Lemyre, E., Micheil Innes, A., Stavropoulos, D.J., Ray, P.N., and Weksberg, R. (2014). High frequency of copy number variations (CNVs) in the chromosome 11p15 region in patients with Beckwith-Wiedemann syndrome. Hum Genet *133*, 321-330.

Beilschmidt, L.K., and Puccio, H.M. (2014). Mammalian Fe-S cluster biogenesis and its implication in disease. Biochimie *100*, 48-60.

Benkel, B.F., and Fong, Y. (1996). Long range-inverse PCR (LR-IPCR): extending the useful range of inverse PCR. Genet Anal *13*, 123-127.

Bochman, M.L., Paeschke, K., and Zakian, V.A. (2012). DNA secondary structures: stability and function of G-quadruplex structures. Nature reviews. Genetics *13*, 770-780.

Bogliolo, M., Schuster, B., Stoepker, C., Derkunt, B., Su, Y., Raams, A., Trujillo, J.P., Minguillon, J., Ramirez, M.J., Pujol, R., *et al.* (2013). Mutations in ERCC4, encoding the DNA-repair endonuclease XPF, cause Fanconi anemia. Am J Hum Genet *92*, 800-806.

Bogliolo, M., and Surralles, J. (2015). Fanconi anemia: a model disease for studies on human genetics and advanced therapeutics. Curr Opin Genet Dev *33*, 32-40.

Broderick, P., Chubb, D., Johnson, D.C., Weinhold, N., Forsti, A., Lloyd, A., Olver, B., Ma, Y.P., Dobbins, S.E., Walker, B.A., *et al.* (2012). Common variation at 3p22.1 and 7p15.3 influences multiple myeloma risk. Nat Genet *44*, 58-61.

Brook, J.D., McCurrach, M.E., Harley, H.G., Buckler, A.J., Church, D., Aburatani, H., Hunter, K., Stanton, V.P., Thirion, J.P., Hudson, T., *et al.* (1992). Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member. Cell *69*, 385.

Brosh, R.M., Jr. (2013). DNA helicases involved in DNA repair and their roles in cancer. Nature reviews. Cancer *13*, 542-558.

Cantor, S., Drapkin, R., Zhang, F., Lin, Y., Han, J., Pamidi, S., and Livingston, D.M. (2004). The BRCA1-associated protein BACH1 is a DNA helicase targeted by clinically relevant inactivating mutations. Proceedings of the National Academy of Sciences of the United States of America *101*, 2357-2362.

Cantor, S.B., Bell, D.W., Ganesan, S., Kass, E.M., Drapkin, R., Grossman, S., Wahrer, D.C., Sgroi, D.C., Lane, W.S., Haber, D.A., *et al.* (2001). BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. Cell *105*, 149-160.

Cantor, S.B., and Guillemette, S. (2011). Hereditary breast cancer and the BRCA1-associated FANCJ/BACH1/BRIP1. Future Oncol *7*, 253-261.

Casper, A.M., Nghiem, P., Arlt, M.F., and Glover, T.W. (2002). ATR regulates fragile site stability. Cell *111*, 779-789.

Chaib, H., Place, C., Salem, N., Chardenoux, S., Vincent, C., Weissenbach, J., El-Zir, E., Loiselet, J., and Petit, C. (1996). A gene responsible for a sensorineural nonsyndromic recessive deafness maps to chromosome 2p22-23. Hum Mol Genet *5*, 155-158.

Chan, N.L., Hou, C., Zhang, T., Yuan, F., Machwe, A., Huang, J., Orren, D.K., Gu, L., and Li, G.M. (2012). The Werner syndrome protein promotes CAG/CTG repeat stability by resolving large (CAG)(n)/(CTG)(n) hairpins. J Biol Chem *287*, 30151-30156.

Chaudhury, I., Sareen, A., Raghunandan, M., and Sobeck, A. (2013). FANCD2 regulates BLM complex functions independently of FANCI to promote replication fork recovery. Nucleic acids research *41*, 6444-6459.

Chen, C.P., Chen, M., Su, Y.N., Tsai, F.J., Chern, S.R., Hsu, C.Y., Wu, P.C., Town, D.D., Lee, D.J., Ma, G.C., *et al.* (2010). Prenatal diagnosis and molecular cytogenetic characterization of de novo partial trisomy 7p (7p15.3-->pter) and partial monosomy 13q (13q33.3-->qter) associated with Dandy-Walker malformation, abnormal skull development and microcephaly. Taiwan J Obstet Gynecol *49*, 320-326.

Chen, J., Jiang, Y., Liu, X., Qin, Z., Dai, J., Jin, G., Ma, H., Wang, S., Wang, X., Hu, Z., *et al.* (2012). Genetic variants at chromosome 9p21, 10p15 and 10q22 and breast cancer susceptibility in a Chinese population. Breast Cancer Res Treat *132*, 741-746.

Chen, Y.H., Jones, M.J., Yin, Y., Crist, S.B., Colnaghi, L., Sims, R.J., 3rd, Rothenberg, E., Jallepalli, P.V., and Huang, T.T. (2015). ATR-mediated phosphorylation of FANCI regulates dormant origin firing in response to replication stress. Molecular cell *58*, 323-338.

Cheung, I., Schertzer, M., Rose, A., and Lansdorp, P.M. (2002). Disruption of dog-1 in Caenorhabditis elegans triggers deletions upstream of guanine-rich DNA. Nat Genet *31*, 405-409.

Choulika, A., Perrin, A., Dujon, B., and Nicolas, J.F. (1995). Induction of homologous recombination in mammalian chromosomes by using the I-SceI system of Saccharomyces cerevisiae. Molecular and cellular biology *15*, 1968-1973.

Chu, W.K., Payne, M.J., Beli, P., Hanada, K., Choudhary, C., and Hickson, I.D. (2015). FBH1 influences DNA replication fork stability and homologous recombination through ubiquitylation of RAD51. Nat Commun *6*, 5931.

Collins, J.R., Stephens, R.M., Gold, B., Long, B., Dean, M., and Burt, S.K. (2003). An exhaustive DNA micro-satellite map of the human genome using high performance computing. Genomics *82*, 10-19.

Collis, S.J., Ciccia, A., Deans, A.J., Horejsi, Z., Martin, J.S., Maslen, S.L., Skehel, J.M., Elledge, S.J., West, S.C., and Boulton, S.J. (2008). FANCM and FAAP24 function in ATR-mediated checkpoint signaling independently of the Fanconi anemia core complex. Molecular cell *32*, 313-324.

Cooper, D.N., Bacolla, A., Ferec, C., Vasquez, K.M., Kehrer-Sawatzki, H., and Chen, J.M. (2011). On the sequence-directed nature of human gene mutation: the role of genomic architecture and the local DNA sequence environment in mediating gene mutations underlying human inherited disease. Hum Mutat *32*, 1075-1099.

Crescenzi, B., Nofrini, V., Barba, G., Matteucci, C., Di Giacomo, D., Gorello, P., Beverloo, B., Vitale, A., Wlodarska, I., Vandenberghe, P., *et al.* (2015). NUP98/11p15 translocations affect CD34+ cells in myeloid and T lymphoid leukemias. Leuk Res *39*, 769-772.

Crowther-Swanepoel, D., Broderick, P., Di Bernardo, M.C., Dobbins, S.E., Torres, M., Mansouri, M., Ruiz-Ponte, C., Enjuanes, A., Rosenquist, R., Carracedo, A., *et al.* (2010). Common variants at 2q37.3, 8q24.21, 15q21.3 and 16q24.1 influence chronic lymphocytic leukemia risk. Nat Genet *42*, 132-136.

De Nicolo, A., Tancredi, M., Lombardi, G., Flemma, C.C., Barbuti, S., Di Cristofano, C., Sobhian, B., Bevilacqua, G., Drapkin, R., and Caligo, M.A. (2008). A novel breast cancer-associated BRIP1 (FANCJ/BACH1) germ-line mutation impairs protein stability and function. Clinical cancer research : an official journal of the American Association for Cancer Research *14*, 4672-4680.

Deakyne, J.S., and Mazin, A.V. (2011). Fanconi anemia: at the crossroads of DNA repair. Biochemistry. Biokhimiia *76*, 36-48.

Deans, A.J., and West, S.C. (2009). FANCM connects the genome instability disorders Bloom's Syndrome and Fanconi Anemia. Molecular cell *36*, 943-953.

Demars, J., and Gicquel, C. (2012). Epigenetic and genetic disturbance of the imprinted 11p15 region in Beckwith-Wiedemann and Silver-Russell syndromes. Clin Genet *81*, 350-361.

Demars, J., Rossignol, S., Netchine, I., Lee, K.S., Shmela, M., Faivre, L., Weill, J., Odent, S., Azzi, S., Callier, P., *et al.* (2011). New insights into the pathogenesis of Beckwith-Wiedemann and Silver-Russell syndromes: contribution of small copy number variations to 11p15 imprinting defects. Hum Mutat *32*, 1171-1182.

Eelen, G., Vanden Bempt, I., Verlinden, L., Drijkoningen, M., Smeets, A., Neven, P., Christiaens, M.R., Marchal, K., Bouillon, R., and Verstuyf, A. (2008). Expression of the BRCA1-interacting protein Brip1/BACH1/FANCJ is driven by E2F and correlates with human breast cancer malignancy. Oncogene *27*, 4233-4241.

Eggermann, T., Spengler, S., Bachmann, N., Baudis, M., Mau-Holzmann, U.A., Singer, S., and Rossier, E. (2010). Chromosome 11p15 duplication in Silver-Russell syndrome due to a maternally inherited translocation t(11;15). Am J Med Genet A *152A*, 1484-1487.

Ellis, N.A., Lennon, D.J., Proytcheva, M., Alhadeff, B., Henderson, E.E., and German, J. (1995). Somatic intragenic recombination within the mutated locus BLM can correct the high sister-chromatid exchange phenotype of Bloom syndrome cells. Am J Hum Genet *57*, 1019-1027.

Enciso-Mora, V., Broderick, P., Ma, Y., Jarrett, R.F., Hjalgrim, H., Hemminki, K., van den Berg, A., Olver, B., Lloyd, A., Dobbins, S.E., *et al.* (2010). A genome-wide association study of Hodgkin's lymphoma identifies new susceptibility loci at 2p16.1 (REL), 8q24.21 and 10p14 (GATA3). Nat Genet *42*, 1126-1130.

Enciso-Mora, V., Hosking, F.J., Kinnersley, B., Wang, Y., Shete, S., Zelenika, D., Broderick, P., Idbaih, A., Delattre, J.Y., Hoang-Xuan, K., *et al.* (2013). Deciphering the 8q24.21 association for glioma. Hum Mol Genet *22*, 2293-2302.

Fan, L., Fuss, J.O., Cheng, Q.J., Arvai, A.S., Hammel, M., Roberts, V.A., Cooper, P.K., and Tainer, J.A. (2008). XPD helicase structures and activities: insights into the cancer and aging phenotypes from XPD mutations. Cell *133*, 789-800.

Fernandez-Lopez, L., Pineiro, E., Marcos, R., Velazquez, A., and Surralles, J. (2004). Induction of instability of normal length trinucleotide repeats within human disease genes. Journal of medical genetics *41*, e3.

Freudenreich, C.H., Stavenhagen, J.B., and Zakian, V.A. (1997). Stability of a CTG/CAG trinucleotide repeat in yeast is dependent on its orientation in the genome. Molecular and cellular biology *17*, 2090-2098.

Frizzell, A., Nguyen, J.H., Petalcorin, M.I., Turner, K.D., Boulton, S.J., Freudenreich, C.H., and Lahue, R.S. (2014). RTEL1 inhibits trinucleotide repeat expansions and fragility. Cell Rep *6*, 827-835.

Fugger, K., Mistrik, M., Danielsen, J.R., Dinant, C., Falck, J., Bartek, J., Lukas, J., and Mailand, N. (2009). Human Fbh1 helicase contributes to genome maintenance via pro- and anti-recombinase activities. The Journal of cell biology *186*, 655-663.

Fugger, K., Mistrik, M., Neelsen, K.J., Yao, Q., Zellweger, R., Kousholt, A.N., Haahr, P., Chu, W.K., Bartek, J., Lopes, M., *et al.* (2015). FBH1 Catalyzes Regression of Stalled Replication Forks. Cell Rep.

Garner, E., and Smogorzewska, A. (2011). Ubiquitylation and the Fanconi anemia pathway. FEBS letters *585*, 2853-2860.

Gatchel, J.R., and Zoghbi, H.Y. (2005). Diseases of unstable repeat expansion: mechanisms and common principles. Nature reviews. Genetics *6*, 743-755.

German, J. (1995). Bloom's syndrome. Dermatol Clin *13*, 7-18.

Glover, T.W., Berger, C., Coyle, J., and Echo, B. (1984). DNA polymerase alpha inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. Hum Genet *67*, 136-142.

Gong, Z., Kim, J.E., Leung, C.C., Glover, J.N., and Chen, J. (2010). BACH1/FANCJ acts with TopBP1 and participates early in DNA replication checkpoint control. Molecular cell *37*, 438-446.

Green, M.R., Treisman, R., and Maniatis, T. (1983). Transcriptional activation of cloned human beta-globin genes by viral immediate-early gene products. Cell *35*, 137-148.

Greenberg, R.A., Sobhian, B., Pathania, S., Cantor, S.B., Nakatani, Y., and Livingston, D.M. (2006). Multifactorial contributions to an acute DNA damage response by BRCA1/BARD1-containing complexes. Genes & development *20*, 34-46.

Gudmundsson, J., Sulem, P., Gudbjartsson, D.F., Masson, G., Petursdottir, V., Hardarson, S., Gudjonsson, S.A., Johannsdottir, H., Helgadottir, H.T., Stacey, S.N., *et al.* (2013). A common variant at 8q24.21 is associated with renal cell cancer. Nat Commun *4*, 2776.

Guenard, F., Labrie, Y., Ouellette, G., Beauparlant, C.J., Bessette, P., Chiquette, J., Laframboise, R., Lepine, J., Lesperance, B., Pichette, R., *et al.* (2007). Germline

mutations in the breast cancer susceptibility gene PTEN are rare in high-risk non-BRCA1/2 French Canadian breast cancer families. Fam Cancer *6*, 483-490.

Gupta, R., Sharma, S., Sommers, J.A., Jin, Z., Cantor, S.B., and Brosh, R.M., Jr. (2005). Analysis of the DNA substrate specificity of the human BACH1 helicase associated with breast cancer. J Biol Chem *280*, 25450-25460.

Gupta, R., Sharma, S., Sommers, J.A., Kenny, M.K., Cantor, S.B., and Brosh, R.M., Jr. (2007). FANCJ (BACH1) helicase forms DNA damage inducible foci with replication protein A and interacts physically and functionally with the single-stranded DNA-binding protein. Blood *110*, 2390-2398.

Hayes, M., and Li, J. (2013). Bellerophon: a hybrid method for detecting interchromosomal rearrangements at base pair resolution using next-generation sequencing data. BMC bioinformatics *14 Suppl 5*, S6.

Ho, C.M., Lin, M.C., Huang, S.H., Huang, C.J., Lai, H.C., Chien, T.Y., and Chang, S.F. (2009). PTEN promoter methylation and LOH of 10q22-23 locus in PTEN expression of ovarian clear cell adenocarcinomas. Gynecol Oncol *112*, 307-313.

Holliday, R., Porterfield, J.S., and Gibbs, D.D. (1974). Premature ageing and occurance of altered enzyme in Werner's syndrome fibroblasts. Nature *248*, 762-763.

Hosoki, K., Ohta, T., Fujita, K., Nishigaki, S., Shiomi, M., Niikawa, N., and Saitoh, S. (2012). Hand-foot-genital syndrome with a 7p15 deletion: clinically recognizable syndrome. Pediatr Int *54*, e22-25.

Houlston, R.S., Cheadle, J., Dobbins, S.E., Tenesa, A., Jones, A.M., Howarth, K., Spain, S.L., Broderick, P., Domingo, E., Farrington, S., *et al.* (2010). Meta-analysis of three genome-wide association studies identifies susceptibility loci for colorectal cancer at 1q41, 3q26.2, 12q13.13 and 20q13.33. Nat Genet *42*, 973-977.

Johnson, R.D., Liu, N., and Jasin, M. (1999). Mammalian XRCC2 promotes the repair of DNA double-strand breaks by homologous recombination. Nature *401*, 397-399.

Joo, W., Xu, G., Persky, N.S., Smogorzewska, A., Rudge, D.G., Buzovetsky, O., Elledge, S.J., and Pavletich, N.P. (2011). Structure of the FANCI-FANCD2 complex: insights into the Fanconi anemia DNA repair pathway. Science *333*, 312-316.

Kamieniak, M.M., Rico, D., Milne, R.L., Munoz-Repeto, I., Ibanez, K., Grillo, M.A., Domingo, S., Borrego, S., Cazorla, A., Garcia-Bueno, J.M., *et al.* (2015). Deletion at 6q24.2-26 predicts longer survival of high-grade serous epithelial ovarian cancer patients. Mol Oncol *9*, 422-436.

Kaneko, Y., Maseki, N., Homma, C., Sakurai, M., Mizutani, S., Takeda, T., Shikano, T., Fujimoto, T., Yaoi, K., and Shimokawa, T. (1988). Chromosome translocations involving band 7q35 or 7p15 in childhood T-cell leukemia/lymphoma. Blood *72*, 534-538.

Kee, Y., and D'Andrea, A.D. (2010). Expanded roles of the Fanconi anemia pathway in preserving genomic stability. Genes & development *24*, 1680-1694.

Kim, H.C., Lee, J.Y., Sung, H., Choi, J.Y., Park, S.K., Lee, K.M., Kim, Y.J., Go, M.J., Li, L., Cho, Y.S., *et al.* (2012). A genome-wide association study identifies a breast cancer risk variant in ERBB4 at 2q34: results from the Seoul Breast Cancer Study. Breast Cancer Res *14*, R56.

Kim, Y., Spitz, G.S., Veturi, U., Lach, F.P., Auerbach, A.D., and Smogorzewska, A. (2013). Regulation of multiple DNA repair pathways by the Fanconi anemia protein SLX4. Blood *121*, 54-63.

Kitao, H., Nanda, I., Sugino, R.P., Kinomura, A., Yamazoe, M., Arakawa, H., Schmid, M., Innan, H., Hiom, K., and Takata, M. (2011). FancJ/Brip1 helicase protects against genomic losses and gains in vertebrate cells. Genes to cells : devoted to molecular & cellular mechanisms *16*, 714-727.

Kitao, S., Lindor, N.M., Shiratori, M., Furuichi, Y., and Shimamoto, A. (1999). Rothmund-thomson syndrome responsible gene, RECQL4: genomic structure and products. Genomics *61*, 268-276.

Kiyotani, K., Mushiroda, T., Tsunoda, T., Morizono, T., Hosono, N., Kubo, M., Tanigawara, Y., Imamura, C.K., Flockhart, D.A., Aki, F., *et al.* (2012). A genome-wide association study identifies locus at 10q22 associated with clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients in Japanese. Hum Mol Genet *21*, 1665-1672.

Kote-Jarai, Z., Jugurnauth, S., Mulholland, S., Leongamornlert, D.A., Guy, M., Edwards, S., Tymrakiewitcz, M., O'Brien, L., Hall, A., Wilkinson, R., *et al.* (2009). A recurrent truncating germline mutation in the BRIP1/FANCJ gene and susceptibility to prostate cancer. Br J Cancer *100*, 426-430.

Kruisselbrink, E., Guryev, V., Brouwer, K., Pontier, D.B., Cuppen, E., and Tijsterman, M. (2008). Mutagenic capacity of endogenous G4 DNA underlies genome instability in FANCJ-defective C. elegans. Current biology : CB *18*, 900-905.

Kuusisto, K.M., Bebel, A., Vihinen, M., Schleutker, J., and Sallinen, S.L. (2011). Screening for BRCA1, BRCA2, CHEK2, PALB2, BRIP1, RAD50, and CDH1 mutations in high-risk Finnish BRCA1/2-founder mutation-negative breast and/or ovarian cancer individuals. Breast Cancer Res *13*, R20.

Kuzminov, A. (2013). Inhibition of DNA synthesis facilitates expansion of low-complexity repeats: is strand slippage stimulated by transient local depletion of specific dNTPs? Bioessays *35*, 306-313.

Leung, C.C., Gong, Z., Chen, J., and Glover, J.N. (2011). Molecular basis of BACH1/FANCJ recognition by TopBP1 in DNA replication checkpoint control. J Biol Chem *286*, 4292-4301.

Levitus, M., Waisfisz, Q., Godthelp, B.C., de Vries, Y., Hussain, S., Wiegant, W.W., Elghalbzouri-Maghrani, E., Steltenpool, J., Rooimans, M.A., Pals, G., *et al.* (2005). The DNA helicase BRIP1 is defective in Fanconi anemia complementation group J. Nat Genet *37*, 934-935.

Levran, O., Attwooll, C., Henry, R.T., Milton, K.L., Neveling, K., Rio, P., Batish, S.D., Kalb, R., Velleuer, E., Barral, S., *et al.* (2005). The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. Nat Genet *37*, 931-933.

Li, D.K., Han, J., Liu, J.B., Jin, G.F., Qu, J.W., Zhu, M., Wang, Y.R., Jiang, J., and Ma, H.X. (2014). Genetic variants at 6p21.1 and 7p15.3 Identified by GWASs of multiple cancers and ovarian cancer risk: a case-control study in Han Chinese women. Asian Pac J Cancer Prev *15*, 123-127.

Li, J.L., Harrison, R.J., Reszka, A.P., Brosh, R.M., Jr., Bohr, V.A., Neidle, S., and Hickson, I.D. (2001). Inhibition of the Bloom's and Werner's syndrome helicases by G-quadruplex interacting ligands. Biochemistry *40*, 15194-15202.

Li, Y., Huang, J., Zhao, Y.L., He, J., Wang, W., Davies, K.E., Nose, V., and Xiao, S. (2007). UTRN on chromosome 6q24 is mutated in multiple tumors. Oncogene *26*, 6220-6228.

Liang, F., Han, M., Romanienko, P.J., and Jasin, M. (1998). Homology-directed repair is a major double-strand break repair pathway in mammalian cells. Proceedings of the National Academy of Sciences of the United States of America *95*, 5172-5177.

Litman, R., Peng, M., Jin, Z., Zhang, F., Zhang, J., Powell, S., Andreassen, P.R., and Cantor, S.B. (2005). BACH1 is critical for homologous recombination and appears to be the Fanconi anemia gene product FANCJ. Cancer cell *8*, 255-265.

Liu, G., Chen, X., Bissler, J.J., Sinden, R.R., and Leffak, M. (2010). Replication-dependent instability at (CTG) x (CAG) repeat hairpins in human cells. Nature chemical biology *6*, 652-659.

Liu, G., Chen, X., Gao, Y., Lewis, T., Barthelemy, J., and Leffak, M. (2012). Altered replication in human cells promotes DMPK (CTG)(n) . (CAG)(n) repeat instability. Molecular and cellular biology *32*, 1618-1632.

Liu, G., Malott, M., and Leffak, M. (2003). Multiple functional elements comprise a Mammalian chromosomal replicator. Molecular and cellular biology *23*, 1832-1842.

Lomonosov, M., Anand, S., Sangrithi, M., Davies, R., and Venkitaraman, A.R. (2003). Stabilization of stalled DNA replication forks by the BRCA2 breast cancer susceptibility protein. Genes & development *17*, 3017-3022.

London, T.B., Barber, L.J., Mosedale, G., Kelly, G.P., Balasubramanian, S., Hickson, I.D., Boulton, S.J., and Hiom, K. (2008). FANCJ is a structure-specific DNA helicase associated with the maintenance of genomic G/C tracts. J Biol Chem *283*, 36132-36139.

Lu, H.W., Huang, M., Wang, J.H., Sun, X.L., and Ke, Y.Q. (2015). CCDC26 rs4295627 polymorphism (8q24.21) and glioma risk: a meta-analysis. Genet Mol Res *14*, 12074-12084.

Lu, T., and Hano, H. (2008). Deletion at chromosome arms 6q16-22 and 10q22.3-23.1 associated with initiation of prostate cancer. Prostate Cancer Prostatic Dis *11*, 357-361.

Lubianca Neto, J.F., Lu, L., Eavey, R.D., Flores, M.A., Caldera, R.M., Sangwatanaroj, S., Schott, J.J., McDonough, B., Santos, J.I., Seidman, C.E., *et al.* (1998). The Bjornstad syndrome (sensorineural hearing loss and pili torti) disease gene maps to chromosome 2q34-36. Am J Hum Genet *62*, 1107-1112.

Lui, W.O., Tanenbaum, D.M., and Larsson, C. (2001). High level amplification of 1p32-33 and 2p22-24 in small cell lung carcinomas. Int J Oncol *19*, 451-457.

Mao, Z., Bozzella, M., Seluanov, A., and Gorbunova, V. (2008a). Comparison of nonhomologous end joining and homologous recombination in human cells. DNA Repair (Amst) *7*, 1765-1771.

Mao, Z., Bozzella, M., Seluanov, A., and Gorbunova, V. (2008b). DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells. Cell Cycle *7*, 2902-2906.

Martino, A., Campa, D., Jamroziak, K., Reis, R.M., Sainz, J., Buda, G., Garcia-Sanz, R., Lesueur, F., Marques, H., Moreno, V., *et al.* (2012). Impact of polymorphic variation at 7p15.3, 3p22.1 and 2p23.3 loci on risk of multiple myeloma. Br J Haematol *158*, 805-809.

Matsuzaki, K., Borel, V., Adelman, C.A., Schindler, D., and Boulton, S.J. (2015). FANCJ suppresses microsatellite instability and lymphomagenesis independent of the Fanconi anemia pathway. Genes & development *29*, 2532-2546.

McMurray, C.T. (2010). Mechanisms of trinucleotide repeat instability during human development. Nature reviews. Genetics *11*, 786-799.

Meier, B., and Gartner, A. (2014). Having a direct look: analysis of DNA damage and repair mechanisms by next generation sequencing. Exp Cell Res *329*, 35-41.

Miret, J.J., Pessoa-Brandao, L., and Lahue, R.S. (1998). Orientation-dependent and sequence-specific expansions of CTG/CAG trinucleotide repeats in Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences of the United States of America *95*, 12438-12443.

Mohaghegh, P., Karow, J.K., Brosh, R.M., Jr., Bohr, V.A., and Hickson, I.D. (2001). The Bloom's and Werner's syndrome proteins are DNA structure-specific helicases. Nucleic acids research *29*, 2843-2849.

Monteilhet, C., Perrin, A., Thierry, A., Colleaux, L., and Dujon, B. (1990). Purification and characterization of the in vitro activity of I-Sce I, a novel and highly specific endonuclease encoded by a group I intron. Nucleic acids research *18*, 1407-1413.

Mori, R., Yoshida, K., Tanahashi, T., Yawata, K., Kato, J., Okumura, N., Tsutani, Y., Okada, M., Oue, N., and Yasui, W. (2013). Decreased FANCJ caused by 5FU contributes to the increased sensitivity to oxaliplatin in gastric cancer cells. Gastric Cancer *16*, 345-354.

Nakanishi, R., Kitao, H., Fujinaka, Y., Yamashita, N., Iimori, M., Tokunaga, E., Yamashita, N., Morita, M., Kakeji, Y., and Maehara, Y. (2012). FANCJ expression predicts the response to 5-fluorouracil-based chemotherapy in MLH1-proficient colorectal cancer. Ann Surg Oncol *19*, 3627-3635.

Nakayama, H. (2002). RecQ family helicases: roles as tumor suppressor proteins. Oncogene *21*, 9008-9021.

Neelsen, K.J., and Lopes, M. (2015). Replication fork reversal in eukaryotes: from dead end to dynamic response. Nat Rev Mol Cell Biol *16*, 207-220.

Nelen, M.R., Padberg, G.W., Peeters, E.A., Lin, A.Y., van den Helm, B., Frants, R.R., Coulon, V., Goldstein, A.M., van Reen, M.M., Easton, D.F., *et al.* (1996). Localization of the gene for Cowden disease to chromosome 10q22-23. Nat Genet *13*, 114-116.

Niedernhofer, L.J., Lalai, A.S., and Hoeijmakers, J.H. (2005). Fanconi anemia (cross)linked to DNA repair. Cell *123*, 1191-1198.

Noda, T., Takahashi, A., Kondo, N., Mori, E., Okamoto, N., Nakagawa, Y., Ohnishi, K., Zdzienicka, M.Z., Thompson, L.H., Helleday, T., *et al.* (2011). Repair pathways independent of the Fanconi anemia nuclear core complex play a predominant role in mitigating formaldehyde-induced DNA damage. Biochem Biophys Res Commun *404*, 206-210.

Ochman, H., Gerber, A.S., and Hartl, D.L. (1988). Genetic applications of an inverse polymerase chain reaction. Genetics *120*, 621-623.

Orazio, N.I., Naeger, C.M., Karlseder, J., and Weitzman, M.D. (2011). The adenovirus E1b55K/E4orf6 complex induces degradation of the Bloom helicase during infection. J Virol *85*, 1887-1892.

Orengo, J.P., Chambon, P., Metzger, D., Mosier, D.R., Snipes, G.J., and Cooper, T.A. (2008). Expanded CTG repeats within the DMPK 3' UTR causes severe skeletal muscle wasting in an inducible mouse model for myotonic dystrophy. Proceedings of the National Academy of Sciences of the United States of America *105*, 2646-2651.

Paeschke, K., Capra, J.A., and Zakian, V.A. (2011). DNA replication through G-quadruplex motifs is promoted by the Saccharomyces cerevisiae Pif1 DNA helicase. Cell *145*, 678-691.

Painter, R.B., and Cleaver, J.E. (1969). Repair replication, unscheduled DNA synthesis, and the repair of mammalian DNA. Radiat Res *37*, 451-466.

Pan, Y., Lui, W.O., Nupponen, N., Larsson, C., Isola, J., Visakorpi, T., Bergerheim, U.S., and Kytola, S. (2001). 5q11, 8p11, and 10q22 are recurrent chromosomal breakpoints in prostate cancer cell lines. Genes Chromosomes Cancer *30*, 187-195.

Paul, V.D., and Lill, R. (2014). SnapShot: eukaryotic Fe-S protein biogenesis. Cell Metab *20*, 384-384 e381.

Pelletier, R., Krasilnikova, M.M., Samadashwily, G.M., Lahue, R., and Mirkin, S.M. (2003). Replication and expansion of trinucleotide repeats in yeast. Molecular and cellular biology *23*, 1349-1357.

Peng, M., Litman, R., Xie, J., Sharma, S., Brosh, R.M., Jr., and Cantor, S.B. (2007). The FANCJ/MutLalpha interaction is required for correction of the cross-link response in FA-J cells. The EMBO journal *26*, 3238-3249.

Petermann, E., Woodcock, M., and Helleday, T. (2010). Chk1 promotes replication fork progression by controlling replication initiation. Proceedings of the National Academy of Sciences of the United States of America *107*, 16090-16095.

Petersen, S., Rudolf, J., Bockmuhl, U., Gellert, K., Wolf, G., Dietel, M., and Petersen, I. (1998). Distinct regions of allelic imbalance on chromosome 10q22-q26 in squamous cell carcinomas of the lung. Oncogene *17*, 449-454.

Pierce, A.J., Johnson, R.D., Thompson, L.H., and Jasin, M. (1999). XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes & development *13*, 2633-2638.

Pike, J.E., Henry, R.A., Burgers, P.M., Campbell, J.L., and Bambara, R.A. (2010). An alternative pathway for Okazaki fragment processing: resolution of fold-back flaps by Pif1 helicase. J Biol Chem *285*, 41712-41723.

Pirzio, L.M., Pichierri, P., Bignami, M., and Franchitto, A. (2008). Werner syndrome helicase activity is essential in maintaining fragile site stability. The Journal of cell biology *180*, 305-314.

Rafnar, T., Gudbjartsson, D.F., Sulem, P., Jonasdottir, A., Sigurdsson, A., Jonasdottir, A., Besenbacher, S., Lundin, P., Stacey, S.N., Gudmundsson, J., *et al.* (2011). Mutations in BRIP1 confer high risk of ovarian cancer. Nat Genet *43*, 1104-1107.

Raghunandan, M., Chaudhury, I., Kelich, S.L., Hanenberg, H., and Sobeck, A. (2015). FANCD2, FANCJ and BRCA2 cooperate to promote replication fork recovery independently of the Fanconi Anemia core complex. Cell Cycle *14*, 342-353.

Raschle, M., Knipscheer, P., Enoiu, M., Angelov, T., Sun, J., Griffith, J.D., Ellenberger, T.E., Scharer, O.D., and Walter, J.C. (2008). Mechanism of replication-coupled DNA interstrand crosslink repair. Cell *134*, 969-980.

Ribeyre, C., Lopes, J., Boule, J.B., Piazza, A., Guedin, A., Zakian, V.A., Mergny, J.L., and Nicolas, A. (2009). The yeast Pif1 helicase prevents genomic instability caused by G-quadruplex-forming CEB1 sequences in vivo. PLoS genetics *5*, e1000475.

Rickman, K.A., Lach, F.P., Abhyankar, A., Donovan, F.X., Sanborn, E.M., Kennedy, J.A., Sougnez, C., Gabriel, S.B., Elemento, O., Chandrasekharappa, S.C., *et al.* (2015). Deficiency of UBE2T, the E2 Ubiquitin Ligase Necessary for FANCD2 and FANCI Ubiquitination, Causes FA-T Subtype of Fanconi Anemia. Cell Rep *12*, 35-41.

Roberts, R., Timchenko, N.A., Miller, J.W., Reddy, S., Caskey, C.T., Swanson, M.S., and Timchenko, L.T. (1997). Altered phosphorylation and intracellular distribution of a (CUG)n triplet repeat RNA-binding protein in patients with myotonic dystrophy and in myotonin protein kinase knockout mice. Proceedings of the National Academy of Sciences of the United States of America *94*, 13221-13226.

Rudolf, J., Makrantoni, V., Ingledew, W.J., Stark, M.J., and White, M.F. (2006). The DNA repair helicases XPD and FancJ have essential iron-sulfur domains. Molecular cell *23*, 801-808.

Sanders, C.M. (2010). Human Pif1 helicase is a G-quadruplex DNA-binding protein with G-quadruplex DNA-unwinding activity. The Biochemical journal *430*, 119-128.

Sawyer, S.L., Tian, L., Kahkonen, M., Schwartzentruber, J., Kircher, M., University of Washington Centre for Mendelian, G., Consortium, F.C., Majewski, J., Dyment, D.A.,

Innes, A.M., *et al.* (2015). Biallelic mutations in BRCA1 cause a new Fanconi anemia subtype. Cancer Discov *5*, 135-142.

Schlacher, K., Christ, N., Siaud, N., Egashira, A., Wu, H., and Jasin, M. (2011). Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. Cell *145*, 529-542.

Schneider, B., Drexler, H.G., and Macleod, R.A. (2011). Molecular breakpoint analysis of chromosome translocations in cancer cell lines by Long Distance Inverse-PCR. Methods Mol Biol *731*, 321-332.

Schwab, R.A., Blackford, A.N., and Niedzwiedz, W. (2010). ATR activation and replication fork restart are defective in FANCM-deficient cells. The EMBO journal *29*, 806-818.

Shaw, D.J., and Harper, P.S. (1989). Myotonic dystrophy: developments in molecular genetics. Br Med Bull *45*, 745-759.

Shigechi, T., Tomida, J., Sato, K., Kobayashi, M., Eykelenboom, J.K., Pessina, F., Zhang, Y., Uchida, E., Ishiai, M., Lowndes, N.F., *et al.* (2012). ATR-ATRIP kinase complex triggers activation of the Fanconi anemia DNA repair pathway. Cancer research *72*, 1149-1156.

Shimada, S., Okamoto, N., Nomura, S., Fukui, M., Shimakawa, S., Sangu, N., Shimojima, K., Osawa, M., and Yamamoto, T. (2013). Microdeletions of 5.5 Mb (4q13.2-q13.3) and 4.1 Mb (7p15.3-p21.1) associated with a saethre-chotzen-like phenotype, severe intellectual disability, and autism. Am J Med Genet A *161A*, 2078-2083.

Sidorova, J.M., Kehrli, K., Mao, F., and Monnat, R., Jr. (2013). Distinct functions of human RECQ helicases WRN and BLM in replication fork recovery and progression after hydroxyurea-induced stalling. DNA Repair (Amst) *12*, 128-139.

Silvestri, V., Rizzolo, P., Scarno, M., Chillemi, G., Navazio, A.S., Valentini, V., Zelli, V., Zanna, I., Saieva, C., Masala, G., *et al.* (2015). Novel and known genetic variants for male breast cancer risk at 8q24.21, 9p21.3, 11q13.3 and 14q24.1: Results from a multicenter study in Italy. Eur J Cancer *51*, 2289-2295.

Somyajit, K., Subramanya, S., and Nagaraju, G. (2012). Distinct roles of FANCO/RAD51C protein in DNA damage signaling and repair: implications for Fanconi anemia and breast cancer susceptibility. J Biol Chem *287*, 3366-3380.

Stoepker, C., Hain, K., Schuster, B., Hilhorst-Hofstee, Y., Rooimans, M.A., Steltenpool, J., Oostra, A.B., Eirich, K., Korthof, E.T., Nieuwint, A.W., *et al.* (2011). SLX4, a coordinator of structure-specific endonucleases, is mutated in a new Fanconi anemia subtype. Nat Genet *43*, 138-141.

Sturzenegger, A., Burdova, K., Kanagaraj, R., Levikova, M., Pinto, C., Cejka, P., and Janscak, P. (2014). DNA2 cooperates with the WRN and BLM RecQ helicases to mediate long-range DNA end resection in human cells. J Biol Chem *289*, 27314-27326.

Subramanian, S., Mishra, R.K., and Singh, L. (2003). Genome-wide analysis of microsatellite repeats in humans: their abundance and density in specific genomic regions. Genome biology *4*, R13.

Suhasini, A.N., and Brosh, R.M., Jr. (2012). Fanconi anemia and Bloom's syndrome crosstalk through FANCJ-BLM helicase interaction. Trends in genetics : TIG *28*, 7-13.

Suhasini, A.N., Rawtani, N.A., Wu, Y., Sommers, J.A., Sharma, S., Mosedale, G., North, P.S., Cantor, S.B., Hickson, I.D., and Brosh, R.M., Jr. (2011). Interaction between the helicases genetically linked to Fanconi anemia group J and Bloom's syndrome. The EMBO journal *30*, 692-705.

Suhasini, A.N., Sommers, J.A., Muniandy, P.A., Coulombe, Y., Cantor, S.B., Masson, J.Y., Seidman, M.M., and Brosh, R.M., Jr. (2013). Fanconi anemia group J helicase and MRE11 nuclease interact to facilitate the DNA damage response. Molecular and cellular biology *33*, 2212-2227.

Tischkowitz, M., and Xia, B. (2010). PALB2/FANCN: recombining cancer and Fanconi anemia. Cancer research *70*, 7353-7359.

Tiscornia, G., and Mahadevan, M.S. (2000). Myotonic dystrophy: the role of the CUG triplet repeats in splicing of a novel DMPK exon and altered cytoplasmic DMPK mRNA isoform ratios. Molecular cell *5*, 959-967.

Uringa, E.J., Youds, J.L., Lisaingo, K., Lansdorp, P.M., and Boulton, S.J. (2011). RTEL1: an essential helicase for telomere maintenance and the regulation of homologous recombination. Nucleic acids research *39*, 1647-1655.

Vannier, J.B., Sandhu, S., Petalcorin, M.I., Wu, X., Nabi, Z., Ding, H., and Boulton, S.J. (2013). RTEL1 is a replisome-associated helicase that promotes telomere and genome-wide replication. Science *342*, 239-242.

Vannier, J.B., Sarek, G., and Boulton, S.J. (2014). RTEL1: functions of a disease-associated helicase. Trends Cell Biol *24*, 416-425.

Virts, E.L., Jankowska, A., Mackay, C., Glaas, M.F., Wiek, C., Kelich, S.L., Lottmann, N., Kennedy, F.M., Marchal, C., Lehnert, E., *et al.* (2015). AluY-mediated germline deletion, duplication and somatic stem cell reversion in UBE2T defines a new subtype of Fanconi anemia. Hum Mol Genet *24*, 5093-5108.

Wagner, J.E., Tolar, J., Levran, O., Scholl, T., Deffenbaugh, A., Satagopan, J., Ben-Porat, L., Mah, K., Batish, S.D., Kutler, D.I., *et al.* (2004). Germline mutations in BRCA2: shared genetic susceptibility to breast cancer, early onset leukemia, and Fanconi anemia. Blood *103*, 3226-3229.

Wan, M., Sun, T., Vyas, R., Zheng, J., Granada, E., and Dubeau, L. (1999). Suppression of tumorigenicity in human ovarian cancer cell lines is controlled by a 2 cM fragment in chromosomal region 6q24-q25. Oncogene *18*, 1545-1551.

Wang, A.T., Kim, T., Wagner, J.E., Conti, B.A., Lach, F.P., Huang, A.L., Molina, H., Sanborn, E.M., Zierhut, H., Cornes, B.K., *et al.* (2015a). A Dominant Mutation in Human RAD51 Reveals Its Function in DNA Interstrand Crosslink Repair Independent of Homologous Recombination. Molecular cell *59*, 478-490.

Wang, D., Zhu, Z.Z., Jiang, H., Zhu, J., Cong, W.M., Wen, B.J., He, S.Q., and Liu, S.F. (2015b). Multiple genes identified as targets for 20q13.12-13.33 gain contributing to unfavorable clinical outcomes in patients with hepatocellular carcinoma. Hepatol Int *9*, 438-446.

Wang, G., and Vasquez, K.M. (2009). Models for chromosomal replication-independent non-B DNA structure-induced genetic instability. Mol Carcinog *48*, 286-298.

Wang, H., Li, Y., Truong, L.N., Shi, L.Z., Hwang, P.Y., He, J., Do, J., Cho, M.J., Li, H., Negrete, A., *et al.* (2014). CtIP maintains stability at common fragile sites and inverted repeats by end resection-independent endonuclease activity. Molecular cell *54*, 1012-1021.

Wang, Y., Leung, J.W., Jiang, Y., Lowery, M.G., Do, H., Vasquez, K.M., Chen, J., Wang, W., and Li, L. (2013). FANCM and FAAP24 maintain genome stability via cooperative as well as unique functions. Molecular cell *49*, 997-1009.

Weinhold, N., Meissner, T., Johnson, D.C., Seckinger, A., Moreaux, J., Forsti, A., Chen, B., Nickel, J., Chubb, D., Rawstron, A.C., *et al.* (2015). The 7p15.3 (rs4487645) association for multiple myeloma shows strong allele-specific regulation of the MYC-interacting gene CDCA7L in malignant plasma cells. Haematologica *100*, e110-113.

White, M.F. (2009). Structure, function and evolution of the XPD family of iron-sulfur-containing 5'-->3' DNA helicases. Biochem Soc Trans *37*, 547-551.

Wikman, H., Sielaff-Frimpong, B., Kropidlowski, J., Witzel, I., Milde-Langosch, K., Sauter, G., Westphal, M., Lamszus, K., and Pantel, K. (2012). Clinical relevance of loss of 11p15 in primary and metastatic breast cancer: association with loss of PRKCDBP expression in brain metastases. PLoS One *7*, e47537.

Wist, E. (1980). Effects of araC and aphidicolin on DNA chain elongation rate in HeLa S3 cells. Experientia *36*, 405-406.

Wu, Y., and Brosh, R.M., Jr. (2009). FANCJ helicase operates in the Fanconi Anemia DNA repair pathway and the response to replicational stress. Current molecular medicine *9*, 470-482.

Wu, Y., Shin-ya, K., and Brosh, R.M., Jr. (2008). FANCJ helicase defective in Fanconia anemia and breast cancer unwinds G-quadruplex DNA to defend genomic stability. Molecular and cellular biology *28*, 4116-4128.

Wu, Y., Sommers, J.A., Khan, I., de Winter, J.P., and Brosh, R.M., Jr. (2012). Biochemical characterization of Warsaw breakage syndrome helicase. J Biol Chem *287*, 1007-1021.

Wu, Y., Suhasini, A.N., and Brosh, R.M., Jr. (2009). Welcome the family of FANCJ-like helicases to the block of genome stability maintenance proteins. Cellular and molecular life sciences : CMLS *66*, 1209-1222.

Xie, J., Guillemette, S., Peng, M., Gilbert, C., Buermeyer, A., and Cantor, S.B. (2010). An MLH1 mutation links BACH1/FANCJ to colon cancer, signaling, and insight toward directed therapy. Cancer prevention research *3*, 1409-1416.

Xue, Y., Li, Y., Guo, R., Ling, C., and Wang, W. (2008). FANCM of the Fanconi anemia core complex is required for both monoubiquitination and DNA repair. Hum Mol Genet *17*, 1641-1652.

Yendamuri, S., Vaporciyan, A.A., Zaidi, T., Feng, L., Fernandez, R., Bekele, N.B., Hofstetter, W.L., Jiang, F., Mehran, R.J., Rice, D.C., *et al.* (2008). 3p22.1 and 10q22.3 deletions detected by fluorescence in situ hybridization (FISH): a potential new tool for early detection of non-small cell lung Cancer (NSCLC). J Thorac Oncol *3*, 979-984.

Ying, S., Hamdy, F.C., and Helleday, T. (2012). Mre11-dependent degradation of stalled DNA replication forks is prevented by BRCA2 and PARP1. Cancer research *72*, 2814-2821.

Youds, J.L., Barber, L.J., Ward, J.D., Collis, S.J., O'Neil, N.J., Boulton, S.J., and Rose, A.M. (2008). DOG-1 is the Caenorhabditis elegans BRIP1/FANCJ homologue and functions in interstrand cross-link repair. Molecular and cellular biology *28*, 1470-1479.

Yu, C.E., Oshima, J., Fu, Y.H., Wijsman, E.M., Hisama, F., Alisch, R., Matthews, S., Nakura, J., Miki, T., Ouais, S., *et al.* (1996). Positional cloning of the Werner's syndrome gene. Science *272*, 258-262.

Zeitouni, B., Boeva, V., Janoueix-Lerosey, I., Loeillet, S., Legoix-ne, P., Nicolas, A., Delattre, O., and Barillot, E. (2010). SVDetect: a tool to identify genomic structural variations from paired-end and mate-pair sequencing data. Bioinformatics *26*, 1895-1896.

Zellweger, R., Dalcher, D., Mutreja, K., Berti, M., Schmid, J.A., Herrador, R., Vindigni, A., and Lopes, M. (2015). Rad51-mediated replication fork reversal is a global response to genotoxic treatments in human cells. The Journal of cell biology *208*, 563-579.

Zeman, M.K., and Cimprich, K.A. (2014). Causes and consequences of replication stress. Nat Cell Biol *16*, 2-9.